Rheumatology
Rheumatology

Seventh Edition

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To our parents (living or of blessed memory) and our spouses, children, and grandchildren

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Rheumatology, Seventh Edition, builds on the success of the previous editions. The most notable change is the addition of Professor Ellen Gravallese as one of the Editors. Professor Gravallese is internationally recognized for her basic and translational work in the study of the immune-mediated inflammatory mechanisms of bone and cartilage destruction in rheumatoid arthritis. Her addition to the Editorial team has refocused our efforts to provide a strong basic and translational science component to the new edition.

Designed to meet the needs of the practicing clinician, this medical reference book provides extensive coverage of rheumatic and musculoskeletal diseases from basic scientific principles to practical points of clinical management in a lucid and logical manner. As stated by Professor Jan Dequeker in his review of the fourth edition, "Rheumatology is the most comprehensive, authoritative rheumatology text designed to meet the complete needs of all practicing and academic rheumatologists as well as arthritis-related health care professionals and scientists interested in disorders of the musculoskeletal system. The edition is firmly grounded on modern medical science, integrating the relevant basic biology with current clinical practice, easily accessible, user-friendly, and a beautifully illustrated color publication." Dr. Harry Brown, in his review of the sixth edition, noted that “[m]y lasting impression of this book was the very same as the first time I opened these two lavish volumes and that was – wow. I would suspect this is the nearest you would get to an encyclopaedia of rheumatology.”

For this new edition, every chapter has been either substantially revised or, in many cases, entirely rewritten, following a rigorous editorial policy to ensure that the content and format of the book remain consistent and meet the highest possible standards. Each chapter has been updated to incorporate a broad range of new information. Seventeen completely new chapters cover basic biomedical and translational science, disease and outcome assessment, including new imaging modalities and early emerging disease, clinical therapeutics, and patient management, including rehabilitation. The text has been streamlined, ensuring that each chapter contains the most critical and current information in the field, while supplemental materials (including extra tables, figures, and bonus text) are conveniently located online. The index has also been improved, making it easier for the reader to find topics of interest.

The production of this edition of Rheumatology has been a greatly enjoyable team effort. We would like to thank the authors who have contributed to this and previous editions of the book, as well as the excellent team at Elsevier, including Jennifer Shreiner, Michael Houston, Nancy Duffy, Ted Rodgers, Bridget Hoette, and Nichole Beard.

We look forward to bringing you the eighth edition in another 4 years.

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We would like to acknowledge the tremendous work of the contributors to this edition of *Rheumatology*, without whom this book would not have been possible. In addition, we would like to recognize our mentors: Drs. Eva Alberman, Ronald J. Anderson, and Laurie Glimcher, and the late Harry Currey, Georg Geyer, Lawrence E. Shulman, Carl Steffen, Alfred D. Steinberg, Mary Betty Stevens, and the late Nathan J. Zvaifler.

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Key Points

- The synovium is a mesenchymal membrane that lines diarthrodial joints, tendon sheaths, and bursae.
- Specialized functions of synovium include nonadherence, control of synovial fluid production and composition, and providing chondrocyte nutrition.
- The normal synovium produces very low levels of proinflammatory cytokines and some antiinflammatory, proresolving cytokines and eicosanoids. In addition, the low levels of expression of RANKL (receptor activator of nuclear factor-κB ligand) with high levels of expression of OPG (osteoprotegerin) result in a low RANKL-to-OPG ratio. This homeostatic balance is likely to be important in preventing osteoclastogenesis in the normal, noninflamed synovium.
- Mesenchymal markers expressed by synovial fibroblasts, such as cadherin-11, endosialin (CD248), and podoplanin (gp58), may be critical for the development of the synovial lining by facilitating cellular organization, compaction, and matrix development. In pathologic settings, they appear to promote cartilage invasion and bone destruction.
- Synovial fibroblasts carry positional and topographic memory that may provide the molecular basis for site-specific differences in the pattern of joint involvement in different rheumatologic diseases.

DEFINITIONS

The study of synovial tissue is of major importance in understanding the pathogenesis of inflammatory arthritis, including rheumatoid arthritis (RA) and seronegative spondyloarthritis (SpA). Despite this, our knowledge of the immunohistochemical architecture of the synovial membrane, particularly in normal subjects, is surprisingly limited, mainly because of the lack of good tissue and cell-specific markers and the difficulty in obtaining synovial tissue in the early as opposed to later stages of disease.

Synovium is the soft tissue lining the spaces of diarthrodial joints, tendon sheaths, and bursae. The term includes both the continuous surface layer of cells (intima) and the underlying tissue (subintima). Whereas the intima is composed of specialized tissue resident macrophages and fibroblasts, the subintima contains blood and lymphatic vessels, a cellular content of both resident fibroblasts and infiltrating cells in a collagenous extracellular matrix (ECM). Between the intimal surfaces is a small amount of fluid, usually rich in hyaluronic (hyaluronic acid). Together, this structure provides a nonadherent surface between tissue elements. Unlike serosal surfaces, which also have nonadherent properties, synovium is derived from ectoderm and does not contain a basal lamina.

In normal subjects, the intimal layer is 20 to 40 µm thick in cross-section, and the areolar subintima can be up to 5 mm in thickness. At many sites, there is no discrete membrane, especially where subintima consists of fat pad or fibrous tissue.

Synovium is often atypical. Intimal cells may be absent. Superficial bursae contain little or no hyaluronan-rich fluid. Ganglia are herniated sacs containing hyaluronan-rich fluid but do not occur at sites of mechanical shearing and do not have a typical intima and so may not be considered really to be synovial tissue. Diseased synovial tissue may lose any recognizable lining structure and may only be definable by its relation to the joint. These variations probably reflect the interplay of several factors in synovial embryogenesis and histogenesis.

EMBRYOLOGY

In the early embryonic limb bud, a central core or blastema that will ultimately form the skeleton appears. Within this core, foci of cartilage appear, each destined to become bone. Blastemal cells around cartilage foci form a perichondrial envelope showing strong CD44 expression. The area where this envelope lies between cartilage elements is known as the interzone, from which the synovium forms. The perichondrium, forming a sleeve around each cartilage element, subsequently invades the cartilage to form bone marrow. Thus, synovial and bone marrow stromal cells come from the same embryonic stock and this is reflected in their transcriptional functional abilities.1,3

Shortly before the joint cavity forms, CD55 expression appears on cells along the joint line1 followed later by vascular cell adhesion molecule 1 (VCAM-1) expression. After cavity formation, the intimal layer also takes on a higher level of expression of CD44 and β1 integrins compared with subintima. Expression of these three markers (CD44, CD55, VCAM-1 [CD106]) confirms the strong similarity between synovial and bone marrow stromal cells.

The mechanism of cavity formation is not fully understood; a working hypothesis implicates interactions between interzone cells bearing CD44 (a hyaluronan receptor) and hyaluronan itself.1,4 Shortly before cavity formation, the cells of the potential joint line show high uridine diphosphoglucose dehydrogenase (UDPGD) activity, which suggests increased hyaluronan synthesis. At the time of cavity formation, high levels of hyaluronan appear along the joint line, saturate CD44, and induce disaggregation; at low concentrations hyaluronan cross-links CD44 molecules on adjacent cells, inducing cell aggregation.

Cavity formation might be expected to require lysis of matrix fibers. However, in human joints, cavity formation is not associated with high local levels of matrix metalloproteinases (MMPs) at the joint line. In fact, matrix fibers appear to run only parallel to the joint line before cavity formation; apoptotic cells found in the interzone at this time are not localized to the joint line and are unlikely to contribute to cavity formation. It appears, therefore, that development of the joint cavity arises more from differential tissue expansion than through loss of solid elements.

STRUCTURE

The microscopic anatomy of synovial tissue was first fully described by Key,7 who divided synovium into three main types on the basis of subintimal structure: fibrous, areolar, and adipose (Fig. 1.1, a to c). He also noted that subintima may be periosteum, perimysium, or even hyaline or fibrocartilage.

Areolar synovium is the most specialized form (see Fig. 1.1, a). It is often crimped into folds, which may disappear when stretched. Less often it carries projections or villi. A more or less continuous layer of cells lies two or three
Adipose synovium occurs as fat pads and within villi (see Fig. 1.1, b). It has a complete intimal cell layer and a superficial net of capillaries. The intima may lie directly on adipocytes, but there is usually a band of collagen-rich substratum, but the deeper tissue is fat. Villi usually have a central arteriole and venule but can be avascular. The amount of fat in villi varies and probably decreases with age, with an increase in fibrous tissue.

Fibrous synovium is more difficult to define, consisting of fibrous tissue such as ligament or tendon on which lies an intermittent layer of cells (see Fig. 1.1, c). Fibrous synovium may be indistinguishable from fibrocartilage, especially in the annular pads found in finger and toe joints.

INTIMAL CELLS

Two types of intimal cells have been defined by electron microscopy, one consistent with a macrophage (type A) and the other with a fibroblast (type B). It is now generally accepted—from immunohistochemical studies and other lines of evidence—that intimal macrophages are true macrophages. However, whether they are derived from circulating precursors from the bone marrow or are derived from fetal macrophages remains unclear. Intimal fibroblasts on the other hand are nonhematopoietic cells and are tissue derived. In normal healthy synovium, synovial fibroblasts are the dominant cell population.

Immunohistochemical and cytochemical methods have superseded electron microscopy as tools for cell identification. Intimal macrophages can be distinguished by their nonspecific esterase (NSE) activity and expression of surface markers such as CD68 and CD163. Often they are CD45 positive as well as expressing podoplanin/gp38 (Fig. 1.2). Intimal fibroblasts show intense activity of the enzyme UDPGD and prominent expression of VCAM-1 and CD55 (complement decay-accelerating factor [DAF]). In most disease states such as RA, intimal cells increase in size and number (Fig. 1.3). This is not just due to hyperplasia but a complex change in cell populations, in terms of both origin and function, which may be dominated by macrophage influx.

**FIG. 1.1** (a) Areolar form of synovium (hematoxylin and eosin [H&E]). (b) Adipose form of synovium (H&E). (c) Fibrous form of synovium (H&E). (Magnification ×200.)

**FIG. 1.2** Stromal markers differentially expressed in the lining and sublining. FAP, Fibroblast activation protein-α.

**FIG. 1.3** Synovium in rheumatoid arthritis (×400) showing a thickened intimal layer containing mainly CD68+ macrophages (red) on the surface and weakly CD55+ fibroblastic cells beneath (blue).
SYNOVIAL MACROPHAGES

Macrophages are present in both the intima and subintima. Intimal macrophages carry typical macrophage lineage markers. They show prominent NSE activity and are strong positive for CD163 and CD68 but less so for CD14 (Fig. 1.4). Macrophages also express the immunoglobulin receptor FcγRIIA. Strong FcγRIIA expression is restricted to a subset of macrophages that correspond closely to sites of macrophage activation in rheumatoid disease: synovial, alveolar, serosal, scleral, and salivary gland; lymphoid tissue and bone marrow; and Kupffer cells. Subintimal macrophages are FcγRIIA null or negative. Macrophages also express Z39Ig, a recently described inducible cell surface receptor linked to the classic complement pathway; Z39Ig expression can occur during macrophage differentiation and induce activation of the transcription factor nuclear factor-κB (NF-κB) and production of matrix-degrading MMP-9.

Macrophages make up a minority of cells in normal intima (Figs 1.4 and 1.5). In disease, the proportion of macrophages increases (see Figs 1.2 and 1.3). Distribution varies, but a common pattern is a superficial layer of macrophages with an intimal phenotype; beneath this a layer of intimal fibroblasts; and further beneath and beyond the limits of the intima, a zone of NSE-weak, strongly CD14+ and FcγRII+ macrophages, associated with venules. The deep, strongly CD14+ cells may be recently recruited cells and the superficial cells tissue resident.

In addition to true macrophages, there may be a small number of antigen-presenting interdigitating dendritic cells in normal synovial intima; these are more frequent in disease with greater overlap of markers, which confounds interpretation. Cells with features of osteoclasts such as expression of tartrate-resistant acid phosphatase and the vitronectin receptor also often appear in inflamed synovium. However, fully typical osteoclasts with calcinon receptors appear to be restricted to pigmented villonodular synovitis and giant cell tumors of tendon sheath.

SYNOVIAL FIBROBLASTS IN THE STROMA

The anatomical term stroma was originally derived from the Greek word describing a platform on which to lie and is used to describe the supporting substrate of a tissue. Its principal role is to maintain the microenvironment required by the parenchyma, the important functional elements of each body system. The stroma includes the cells of mesenchymal origin; the nerves, the vessels, and the epithelia that reside in a tissue in steady state; and the extracellular matrices and fluids that these cells produce. Traditionally, the diversity of stromal cells, and in particular fibroblast phenotype and function and their roles beyond those of space filling and ECM homeostasis, has been underplayed in the synovium. We now know that these cells vary phenotypically at different anatomical sites and contribute significantly to the identity of individual tissues, providing the so-called ‘stromal postcode.’ Furthermore, it is known that rather than acting as a bystander to the body’s protective mechanisms and to disease processes, the fibroblast is capable of actively participating and indeed orchestrating inflammation and immunity.

The fibroblast communicates with resident and infiltrating cells via cytokines and cell contact dependent mechanisms, playing a central role in the pathogenesis of synovial pathology.

The synovial intima contains cells that are adapted to hyaluronan production. CD68-negative intimal fibroblasts express high enzymatic activity for UDPGD. UDPGD converts UDP glucuronate into UDP glucuronone, one of the two substrates required by hyaluronan synthase for hyaluronan polymer assembly. Unlike the activity of many other enzymes, UDPGD activity in intimal fibroblasts is reduced, rather than enhanced, in diseased tissue. Synovial intimal fibroblasts express CD55 (see Fig. 1.5), a feature distinguishing them from intimal macrophages.

Cells disaggregated from inflamed synovium and grown in tissue culture display fibroblast characteristics and ramifying processes with production of high levels of MMPs. It is not known whether they derive from intimal or subintimal cells. In tissue sections, immunoreactivity for collagenase and gelatinase is patchy and not necessarily confined to the intima.

Synovial intimal fibroblasts also show prominent expression of several adhesion molecules, including VCAM-1, intercellular adhesion molecule 1, CD44, and β1 and β2 integrins. Expression of VCAM-1 (Fig. 1.6) is particularly unusual, being absent from most other normal fibroblast populations, but CD44 and β1 integrins are present at lower levels. The role of VCAM-1 with respect to intimal fibroblasts is puzzling, reflecting its embryologic similarities to bone marrow fibroblasts, which are also VCAM-1 positive.

Expression of VCAM-1 may modulate cell trafficking because its ligand, αvβ3 integrin, is present on mononuclear leukocytes but not granulocytes. Intimal fibroblasts may allow transmigration of polymorphs but not mononuclear cells. The expression of podoplanin (which in the setting of neoplasia is associated with poor prognosis and metastatic disease) has been shown to be highly expressed in RA synovial fibroblasts with their attendant migratory and invasive potential. Under inflammatory conditions, fibroblasts can act as organ-specific sentinel cells, where they play a role in the switch from acute resolving to chronic persisting. In addition to contributing to the recruitment and emigration of inflammatory cells to and out of the joint, they modulate the survival and retention of infiltrating leukocytes. Interestingly, new data raise the possibility of epigenetically programmed aggressive fibroblasts ‘spreading’ arthritis from inflamed to uninfamed joints in the early stages of arthritis but at the same time offering the possibility of specifically targeting stromal subpopulations of choice.

The expression of two other surface molecules by synovial fibroblasts is noteworthy. Complement receptor 2 (CR2, CD21) is not expressed by normal intimal fibroblasts but can be induced on synovial fibroblasts in culture, in contrast to other fibroblast populations. DAF, VCAM-1, and CR2 are all involved in B-lymphocyte survival, as is a bone marrow stromal cell marker, BST-1, reported to be expressed on fibroblasts in rheumatoid, but not normal, synovial intima. Other molecules associated with bone marrow stromal

![Image 1.4](image1.png) Synovial macrophages. Normal synovium (x200) stained for CD68+ macrophages (red).

![Image 1.5](image2.png) Normal synovium (x200) stained for CD55+ fibroblasts, which are the predominant cell in the normal synovium intimal layer (contrast with Fig. 1.3).

![Image 1.6](image3.png) Normal synovium (x200) stained for vascular cell adhesion molecule 1.
cells such as the chemokine stromal cell–derived factor-1 (CXCL12) and bone morphogenetic proteins and their receptors,41-43 are expressed by synovial fibroblasts under various conditions. Moreover, lubricin, otherwise known as superficial zone protein, a glycoprotein found in synovium and the superficial zone of articular cartilage,44 derives from the same gene as megakaryocyte-stimulating factor. A defect of this gene leads to CACP (camptodactyly arthropathy coxa vara pericarditis) syndrome. As indicated earlier, these patterns of gene expression may reflect a common embryologic origin for synovial and bone marrow stromal cells.

Self-renewing mesenchymal stem cells that compare favorably with bone marrow–derived mesenchymal stem cells in terms of their ability to differentiate into bone, cartilage, and adipose tissue have been isolated from the normal synovium; it is unclear which component of the synovial membrane is home to these cells,45,46 but expression of the mesenchymal stromal cell marker CD248 in the sublining layer (see Fig. 1.7) suggests that they may derive from this anatomical compartment.47

**INTIMAL MATRIX**

Intimal matrix has an amorphous or fine fibrillar ultrastructure. It is poor in type I collagen but contains minor collagens III, IV, V, and VI as well as laminin, fibronectin, and chondroitin-6-sulfate–rich proteoglycan, which, with collagen IV, are components of basement membrane; however, the basement membrane is conspicuous by its absence beneath the intimal layer. The looser structure of intimal matrix may be explained by the absence of entactin, which links other components in basement membrane together. Intimal microfibrils are of two types: fibrillin-1 microfibrils form a basketwork around cells, and collagen VI microfibrils form a uniform mesh.

Intimal matrix contains large amounts of hyaluronan (Fig. 1.8), which tails off 20 to 50 µm deep; this possibly indicates diffusion from the surface toward clearing lymphatics.

**VASCULAR NET**

A rich microvascular net lies beneath the synovial surface.10,11 Capillaries (prominent in children and decreasing with age) occur just below or within the intima (Fig. 1.9). Some capillaries are fenestrated, and fenestrae tend to face the tissue surface; 50 to 100 µm beneath the surface, small venules are prominent. About 200 µm beneath the surface, larger venules, together with arterioles and lymphatics (Fig. 1.10),12 form an anastomosing quadrilateral array. Vessels with lymphatic staining characteristics are prominent in RA synovium. It has been proposed that failure of lymphatic drainage of synovial fluid is a cause of villous proliferation in RA synovial tissue. If this is correct, it is likely to be due to overloading of existing lymphatic channels with hyaluronan–rich extracellular fluid and leukocytes rather than a lack of lymphatic channels.12

Apart from the fenestration of superficial capillary endothelial cells, there is little evidence of specialization in synovial endothelium. Endothelial cells enlarge in inflamed tissue, and microvascular proliferation can occur, but...
these events are common to inflammation at many sites. Tissue-specific adhesion molecules, or addressins, have been sought in the synovium, but nothing conclusive has been found unlike the case in the skin and gut, both epithelial organs.

**CELL ORIGINS AND RECRUITMENT**

Evidence to date indicates that both intimal and subintimal macrophages derive from bone marrow via circulating monocytes, many of which probably arrive through subintimal venules and migrate to the intima. Whether tissue resident macrophages also contribute remains unclear, but recent studies suggest that nonclassical monocytes or macrophages can contribute to the persistence or resolution of arthritis.\(^5\)

Intimal fibroblasts are thought to arise by division within synovium. They might be a discrete self-replicating population, distinct from subintimal fibroblasts, but several pieces of evidence argue against this. Rates of cell division within the intima are very low, even in disease. After arthroplasty or synovectomy, intimal cells—likely replaced from the subintima rather than arising from intimal rests—reappear and express CD55, UDPGD, and VCAM-1. Disaggregated and cultured synovial fibroblasts lose VCAM-1 and CD55 expression, but the majority, apparently including cells of subintimal origin, readily express these markers after cytokine stimulation, in contrast to fibroblasts of dermal or subcutaneous origin. These findings suggest that synovial fibroblasts, in both the intima and subintima, belong to a specialized population with a propensity to express VCAM-1 and CD55 and are more similar to bone marrow than skin fibroblasts.\(^5\)

Two studies\(^5\) have demonstrated the range of cells that can be found in the synovial subintima. CD3+ T cells, including CD4+, CD8+, and memory T cells, can be found within the normal synovial tissue; although they are likely to be simply trafficking through the normal synovium, their role, if any, in the homeostasis of synovial tissue is unknown. It is also possible to detect B cells, plasma cells, and granzyme B+ cells in normal synovium, although they are present in small numbers.

Although production of inflammatory cytokines, including interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α),\(^9\) can be detected in normal synovial tissue, expression levels are far lower than those seen in inflamed synovial tissue such as in RA. The amount of antinflammatory cytokine production, at least in the case of IL-1 receptor antagonist (the naturally occurring inhibitor of IL-1), IL10, and other prosolving factors is far greater than the amount of inflammatory cytokine seen (Fig. 1.11).\(^5\) This would achieve the desired result of suppressing an inflammatory process in the normal synovial tissue. Similarly, the amount of RANKL (receptor activator of NF-κB ligand, a latent complex for the development of osteoclasts) seen in normal synovial tissue is low.\(^5\) The net result of this is to suppress the formation of osteoclasts within the normal synovium and preserve homeostasis within the normal joint.

**FUNCTION**

The functions of synovial tissue have proven to be remarkably difficult to define.\(^3\) Similar to other soft connective tissue, synovium provides a deformable packing that allows movement of adjacent, relatively nondeformable tissues. The difference between synovium and other soft connective tissue is that it allows most of the movement to occur between rather than within tissues. Areolar synovium may also have specialized viscoelastic properties for coping with the stretching, rolling, and folding it undergoes during joint movement.

Functions of the tissue relating to the synovial cavity may be considered to be the following:

- Maintenance of an intact nonadherent tissue surface 
- Lubrication of cartilage 
- Control of synovial fluid volume and composition 
- Nutrition of chondrocytes within joints

**MAINTENANCE OF THE TISSUE SURFACE**

Synovial surfaces must be nonadherent to allow continued articular movement. Animal models suggest that production of hyaluronan by intimal fibroblasts may be important in inhibiting adhesion.\(^7\) Plasminogen activator and DAF from intimal fibroblasts also inhibit fibrin formation and scarring. To retain synovial fluid, the intimal matrix consists of a fibrous mat of a particular porosity that allows free exchange of crystalloids and proteins but inhibits rapid transit of the viscous hyaluronan solution that is an important component of the fluid. The vasculature is likely to be important in both intimal cell nutrition and recruitment of new cells. New macrophages are derived from blood monocytes that are thought to enter the tissue through venules, and perivascular fibroblasts may provide the main pool of intimal fibroblast precursors.

**LUBRICATION**

The ability of synovial fluid to lubricate cartilage surfaces depends on the presence of glycoproteins, especially a glycoprotein known as both *lubricin* and *superficial zone protein* because of its localization to the surface of synovium and cartilage.\(^4\)

Whatever the precise forces acting on fluid volume, the presence of hyaluronan is likely to be the main factor responsible for retaining a constant volume of fluid during exercise.\(^4\) This fluid is probably important as a cushion for synovial tissue and as a reservoir of lubricant for cartilage. It is likely that mechanical stimulation of intimal fibroblasts dictates the rate of synthesis and exportation of hyaluronan into the synovial fluid compartment. Thus, when synovial fluid volume is high, reduced mechanical stresses on intimal fibroblasts result in a reduced rate of hyaluronan production and vice versa.

Two distinct mechanisms create joint effusions. When synovium is mechanically irritated by worn bone and cartilage, the composition of the fluid remains reasonably normal. Excessive production of hyaluronan by intimal fibroblasts stimulated by fractional forces retains plasma dialysate in the synovial cavity; in synovitis, the effusion is an accumulation of exudate similar to a pleural effusion (i.e., an overspill from the inflammatory edema in synovial tissue created by increased vascular permeability). Recent theories about possible low-grade inflammatory and immune reactions contributing to the pathogenesis of osteoarthritis suggest that these two mechanisms of effusion development may not be as distinct as originally thought.\(^3\) Additionally, recent proteomic evidence suggests that the increased vascular permeability as seen in inflammation may be related not only to an increase in interendothelial gaps but also to glycocalyeal damage and aquaporin upregulation.\(^46\)

**CHONDROCYTE HEALTH AND NUTRITION**

The synovium provides the major structure that aids chondrocyte nutrition. In normal joints, a surprisingly large proportion of hyaline cartilage lies within 50 µm of a synovial surface. In any one position, only a small proportion of cartilage is opposed to the other articular surface, and synovium packs most of the space between less congruent areas. In immature joints, the incomplete subchondral plate may contribute to nutrition, but in adult joints, this route is unlikely to be significant. Nutrition of areas of cartilage that do not come into close contact with synovium (concave articular surfaces in particular) must take an indirect route. Although a small proportion of nutrition may be imparted by smearing of a thin film of fluid over these surfaces during movement, indirect routes through cartilage matrix and the apposed articular cartilage may be more important.\(^4\)

Diarthrodial joints (in both cartilage and synovium) have been found to express high levels of transforming growth factor-β (TGF-β), a latent complex that requires activation to induce a biologic response. Recent in vivo experiments suggest that shearing of synovial fluid because of physiologic joint motion may play an important role in TGF-β activation, which may be essential to maintain the biochemical content and structural integrity of healthy cartilage.\(^32\)

Even though the blood vessels in synovium provide the most direct route for cartilage nutrition, there is no evidence that they are structurally adapted to this function. The fenestrae seen in superficial capillaries are present in

![FIG. 1.11 Normal synovium (x200) stained with an antibody to detect the interleukin-1 receptor antagonist.](image)
SYNOVIAL JOINTS ARE INVOLVED IN SEVERAL IMMUNOLOGIC AND INFLAMMATORY DISORDERS, INCLUDING RA, SYSTEMIC LUPUS ERYTHEMATOUS, AND SERONEGATIVE SPAP. PERHAPS THE MOST IMPORTANT REASON FOR STUDYING THE BIOLOGY OF THE SYNOVIAL TISSUE IS TO-obtain insight into which immunopathologic processes are likely to be suitable therapeutic targets in inflammatory arthritis and in particular whether the synovial fibroblast is a therapeutic target. 2, 3

Autoimmune responses to synovial antigens might theoretically occur, but despite considerable effort, evidence for a specific synovial antigen is lacking. Moreover, associated targeting of other tissues such as pericardium or uveal tract requires an explanation. Few, if any, synovium-specific antigens are known, and when rheumatic disorders are associated with autoantibodies, the antigens involved are ubiquitous.

Understanding the microarchitecture of the normal synovium, including the wide range in microscopic appearances, cellular infiltrates, and production of cytokines, enzymes, and other biologically relevant proteins, assists in understanding the relevant changes in synovial tissue architecture and immunopathology in disease states. Although the architecture of the normal synovium is not as homogeneous as previously portrayed, consistencies across the broad spectrum of normal synovial tissues can be contrasted with those seen in chronically inflamed synovial tissue. The marked increase in synovial lining layer thickness with a reversal of the normal ratio of type A to type B cells, which favors type B cells in normal synovium and type A cells in RA, is an example of this. Numerous other examples can be given, including the changes in subintimal cell content and cytokine and chemokine production, vascular and lymphatic changes, and production of MMPs and stimulators of osteoelastic formation.

A recent study in a mouse model of arthritis has also raised the possibility of cadherin-11 expression on synovial fibroblasts as a potential therapeutic target in the treatment of RA 25-28; inhibition of cadherin-11 interactions in this model interfered with both synovial inflammation and cartilage erosion by pannus without having any effect on bone erosion (predominantly osteoclast dependent) or immunosuppression. In this model, inflammation could be reduced substantially by antibodies to cadherin-11 or a cadherin-Fc fusion protein. Furthermore, cadherin-11 expression has been found to promote invasive behavior of fibroblasts and is increased by IL-17 and TNF-α, cytokines very relevant in RA pathophysiology. 29-32

SUMMARY

Despite the biologic importance of understanding how the synovium responds to damage and drives inflammation, remarkably little is known about how stromal cells (as opposed to leukocytes) change during synovial development and inflammation. Difficulties in accessing synovial tissue from normal subjects and patients with early disease and the lack of good cell markers have proved to be obstacles to such work. However, synovial stromal cells are a functionally heterogeneous group with some displaying proinflammatory and destructive properties but others being immune regulatory and helping facilitate tissue repair. This has led to a dilemma: Which stromal cells should be targeted, and which should be retained? A clear understanding of the biology and significance of synovial tissue biology is therefore essential to provide a coherent rationale for targeting stromal cells in the future treatment of patients with arthritis.

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REFERENCES


Articular cartilage has key roles in the function of joints. A major function is to take up and distribute load such that a given point of the underlying bone can handle very high strain. Another role of cartilage is to provide low-friction movement. One key feature of joint diseases is deterioration of bone can handle very high strain. Another role of cartilage is to provide low-friction movement. One key feature of joint diseases is deterioration of bone properties important for load distribution and dissipation. The fibers contain other matrix proteins (e.g., those bound at their surface) that mediate interactions with other tissue structures, including neighboring fibers, which enhances their mechanical qualities.

Cartilage ECM contains growth factors and proenzymes that are sequestered by binding to matrix macromolecules, and these substances can be released upon degradation of the carrier molecules. As a result of degradation of cartilage matrix, the fragments formed are released into surrounding fluids and can be used as indicators of the ongoing process, the so-called molecular marker technology. Fragments of ECM components can activate innate immune responses such as complement.


### Key Points

- The cells in cartilage maintain function of the extracellular matrix (ECM) via controlled turnover in response to minor damage caused by fatigue and altered load by removing malfunctioning matrix constituents by breakdown and producing new ones to achieve repair.
- The ECM of cartilage is different from other tissues in that it is a network in which globular domains interact with other molecules.
- Matrix networks with collagen as the major constituent provide the tensile properties essential for load distribution and dissipation. The fibers contain other matrix proteins (e.g., those bound at their surface) that mediate interactions with other tissue structures, including neighboring fibers, which enhances their mechanical qualities.
- Cartilage ECM contains growth factors and proenzymes that are sequestered by binding to matrix macromolecules, and these substances can be released upon degradation of the carrier molecules.
- As a result of degradation of cartilage matrix, the fragments formed are released into surrounding fluids and can be used as indicators of the ongoing process, the so-called molecular marker technology.
- Fragments of ECM components can activate innate immune responses such as complement.

### OVERALL TISSUE ORGANIZATION

The part of the matrix closer to cells, the territorial matrix, has a somewhat different composition and structure than the matrix at some distance, the interterritorial matrix (Fig. 2.2) (for references, see Heinegård and colleagues). Examples of components found in both compartments are collagen type II fibers and aggrecan; in contrast, collagen type VI is found particularly in the territorial matrix, and cartilage oligomeric matrix protein (COMP) and cartilage intermediate layer protein (CILP) are primarily found in the interterritorial matrix of normal cartilage. There is also a difference in the composition of cartilage from the superficial to deep layers. In the superficial layer, collagen fibers are thinner and arranged in parallel with the surface of the tissue; in the deeper layer, the fibers are thicker and arranged perpendicular to the surface, with a transition zone in between them (Fig. 2.1; also see Fig. 2.1). The superficial part of cartilage is enriched with a number of noncollagenous molecules, notably lubricin and asporin. At the same time, other molecules are much less abundant in this part of the tissue, as exemplified by aggrecan, which is particularly enriched toward the deeper parts of the tissue. Certain molecules such as CILP are found primarily in the middle portions of articular cartilage. Although a number of cartilage components have been described in recent years, our understanding of what specific requirements and functions are met by molecules with such a restricted localization in tissue is still very limited.

Different types of cartilage have markedly different compositions. Articular cartilage has major similarities, yet multivariate analyses of proteomics data have demonstrated differences between knee and hip articular cartilage.

### AGGREGAN

The major structure of aggrecan, illustrated in Fig. 2.3, consists of approximately 100 chondroitin sulfate glycosaminoglycan chains, each built from a disaccharide unit that is repeated some 50 times but with extensive variability. Each disaccharide contains uronic acid with a negatively charged carboxyl group and an N-acetylgalactosamine with a sulfate in either the 4 or the 6 position. Each chain will therefore contribute around 100 negatively charged groups. The glycosaminoglycans are linked to a serine residue of the protein core of the proteoglycan via their reducing terminal end. The chains are clustered, and clustering differs between the two regions referred to as CS domain 1 and CS domain 2 (see Fig. 2.3). There is an additional glycosaminoglycan, keratan sulfate, that has a disaccharide building block of galactose and an N-acetylgalactosamine with a sulfate in the 6 position. These chains are shorter and particularly enriched closer to the N-terminal end of the protein core, in the so-called keratan sulfate–rich region. The aggrecan molecule is composed of some 30 such chains.

The proteoglycan core protein contains globular domains flanking the three domains carrying glycosaminoglycan chains. The one most N-terminal, the hyaluronic-binding domain (G1 globule), contributes specific high-affinity binding of the aggrecan molecule to hyaluronan (see below). After a short interglobular domain there is a second globular domain (G2) that has structural similarities to the G1 globule but does not bind hyaluronan and has no known function. In the very C-terminal end of the aggrecan molecule, there is a G3 globule with a lectin homology domain; it contributes by binding to other proteins (e.g., fibulins and tenascins), which themselves can form molecular complexes involving several such molecules (see Fig. 2.2). Recent, mutations
Collagen fibrillogenesis is a multistep process. Fibers formed vary in size.

FIG. E2.1 The organization of collagen fibers at different distances from the surface of articular cartilage is shown, as well as differences in fibril diameters between the pericellular, territorial, and interterritorial compartments.
that abolish binding of the G3 domain to the fibulins and tenascins have been shown to lead to familial osteochondritis dissecans.3

Many aggrecan molecules will bind to a single, very long strand of hyaluronan, thereby forming large aggregates containing more than 100 glycosaminoglycan chains, each with some 100 negatively charged groups. These charges are thus fixed in the tissue, and the presence of counter ions results in an osmotic environment that retains water and has an essential role in cartilage function by resisting compression and distributing load. The interaction of aggrecan via its G1 globe with hyaluronan is stabilized by the link protein having structures similar to the hyaluronan-binding domain of aggrecan. This link protein will bind to this domain of the proteoglycan, as well as to hyaluronan.

In normal cartilage turnover, as well as in pathology, the aggrecan molecule is cleaved by enzymes called aggreganases (i.e., ADAMTS-4 [a disintegrin and metalloproteinase with thrombospondin motifs] and ADAMTS-5)12,13 (see Fig. 2.3). One site of this cleavage is in the interglobular domain between the hyaluronan-binding G1 globe and the G2 globe. The cleavage occurs between the amino acids EGE and ARG (for references, see elsewhere6). The cleavage occurs in the domain carrying the chondroitin sulfate chains results in shortening of the aggrecan with ensuing decreased number of fixed charged groups (see Fig. 2.3). With aging, shorter aggrecan molecules accumulate, the extreme being hyaluronan-binding domain with no glycosaminoglycan-binding structures remaining.8 Indeed, in adults, a major proportion of the aggrecan molecules found in the interterritorial matrix at a distance from the cells do not contain the G3, C-terminal globular lectin homology domain (Asberg and Heinegård, unpublished observation). On the other hand, the molecules in the territorial matrix close to the cells contain this domain, probably a result of the gradual turnover of the aggrecan molecules. At the same time, there is substantial accumulation of G1 domain, apparently retained by being bound to hyaluronan.9 This fragment can be identified in tissue either via its new C-terminal as represented by an -EGE373-COOH sequence formed through cleavage by aggrecanase or via a further matrix metalloproteinase (MMP) cleavage that forms a C-terminal -PEN341-COOH sequence.10

Even from early experiments by Thomas11 and Fitton-Jackson and collaborators,12 it was clear that chondrocytes have a remarkable capacity to replace aggrecan molecules removed from tissue by the use of enzymes cleaving hyaluronan. It appears that a normal chondrocyte should be able to replace even large amounts of proteoglycans lost unless suppressed by cytokines such as IL-1 and TNF-α.

There is increasing evidence that the aggrecan degradation encountered in early joint disease, such as OA, is effectively counterbalanced by increased synthesis and deposition of the molecule such that no overall loss takes place.13,14

COLLAGEN FIBRILLAR NETWORKS

A major function of the ropelike collagen fiber networks in cartilage is to provide tensile strength and distribute the load so that excessive local force is not applied to the underlying bone. One type of fiber contains a core of collagen type II with a minor constituent of collagen type XI connected via a number of molecules bound at its surface.15

The other fibrillar network contains a core of collagen type VI that forms a filamentous network primarily in the territorial matrix. This network is connected by a set of linker molecules to other molecules in the matrix, including collagen type II fibers and the major proteoglycans. These networks are discussed separately.

FIBERS WITH COLLAGEN TYPE II AS THE MAIN CONSTITUENT

Collagen type II and collagen type XI

The major fibrillar network in cartilage contains primarily collagen type II with a minor constituent of collagen type XI.15 A major feature of both these types of collagen is that the molecule forming the fibers is made up of three parallel, tightly associated polypeptide chains forming a very stable triple
helix, the collagen molecule. It is extremely asymmetric—300 nm long and 1.5 nm in diameter. An amino acid quite unique for collagen is hydroxyproline, which is essential for stability of the molecule because of the hydrogen bonds formed to the hydroxy group. Collagen type II is produced as a procollagen that does not form fibrils until the propeptides at both ends of the C- and N-termini are cleaved off (see Fig. e2.1). After this cleavage, the molecules form fibers by interactions with other collagen molecules such that a large part of the surface of the molecule is engaged and the collagen molecules are positioned so that they form a so-called quarter stagger arrangement in relation to one another.

The assembly process is regulated by a number of molecules in the matrix, including collagen type XI. It appears that the dimensions of the fiber formed depend on the relative proportion of collagens type II and XI, with the typical ratio being on the order of 50:1. This may depend on the presence of a central core of microfibrils of collagen type II and XI that direct assembly of the fiber.19

Collagen fibers in the superficial and deep layers of articular cartilage are different in dimension and direction. Thus, whereas fibers in the superficial layer of articular cartilage are thin and run in parallel, the thicker fibers in the deeper parts of cartilage run perpendicular to the surface. In the transition zone layer, fibers run at an angle (see Fig. e2.1).17 The organization can be seen as Benninghoff arcades on polarized light microscopy.

Collagen fiber formation is influenced by a number of matrix molecules, such as decorin, asporin, fibromodulin, COMP, and a special variant of an oversulfated chondroitin sulfate chain. In several of these cases, the molecules are also retained bound at the surface of the collagen fiber. This is particularly evident for collagen type IX, which has part of the molecule extending out from the fiber. These molecules appear to have roles in providing sites for interaction with other matrix molecules, including fibers other than those to which the protein is bound (see Fig. 2.2).

An important feature of the collagen fiber network is that the interactions become sealed by covalent cross-link formation after the fibers are assembled outside the cell. This cross-linking depends on the oxidation of lysine and hydroxylysine residues by lysyl oxidase to provide an aldehyde function that forms a Schiff base with a neighboring lysine amino group. These are then rearranged to become stable pyridinoline groups that cross-bridge between the molecules and within the molecules of a fiber. These cross-links are important for mechanical stability of the collagen. Because they are not metabolized on degradation of the collagen, they eventually end up in urine and can be measured as indicators of collagen breakdown.20

**Collagen type XI**

This fibril-forming collagen is an integral component of the fibers forming the main network in cartilage. Collagen type XI consists of a major triple-helical portion, similar in size to that of collagen types I and II, but in contrast to these collagens, the N-terminal propeptides are retained with the molecule incorporated into the fiber. Some reports have indicated that the retained N-terminal parts are exposed at the surface of the fibers, with collagen type II being the major constituent and the major triple-helical portion being located more centrally in the fiber.19 Collagen type XI together with collagen type II appears to form the initial assembly of microfibrils that regulate further assembly of the cartilage collagen fiber, at least in skeletal morphogenesis.18 Interestingly, collagen type XI forms cross-links to primarily other collagen type XI molecules.20 There are examples of mutations in collagen type XI chains with ensuing major growth disturbances, thus indicating a role in cartilage growth and stability.21

**Collagen type IX**

This molecule is a member of the FACIT collagens (fibril-associated collagens with interrupted triple helices) and is found in tissue bound at the surface of the fibrils, with collagen type II being the major constituent. Collagen type IX contains three different α chains with three triple-helical domains (col1, col2, and col3), each surrounded by a noncollagenous domain (NC1, NC2, NC3, and NC4). The NC4 domain with its adjacent col3 triple helix protrudes from the fibers and is available for interactions with other molecules in the ECM, as schematically illustrated in Fig. 2.2.22 Examples of such ligands are COMP and the tyrosine sulfate domain of fibromodulin. Collagen type IX often contains a chondroitin sulfate side chain bound at the NC3 domain. Its role in function of the collagen is not known.

Functionally, collagen type IX has been shown to interact with matrilins, COMP, and in particular, collagen type II. The collagen is actually covalently cross-linked to collagen type II in the fibers of adults.23 When collagen type IX is added in vitro to fibril-forming systems of collagen type II, assembly and fiber formation are retarded.

Mutations in collagen type IX lead to severe growth disturbances, such as pseudoachondroplasia or multiple epiphyseal dysplasia. Some of these
Moieties regulating collagen fiber assembly

The dimensions and orientation of collagen fibers in tissue vary between different layers of articular cartilage. Moreover, fibers in the territorial matrix close to cells are thinner and have similar dimensions in different layers of cartilage. In contrast, fibers in the interterritorial matrix are thicker and have larger and more variable diameters in the deeper layers. This regulation of fiber diameter is achieved by a number of macromolecules that bind to collagen. The exact role of individual molecules in achieving the final dimensions and orientation of the fibers is not clear, although there are a number of examples in which inactivation of individual genes of the involved proteins leads to altered dimensions of collagen fibrils. It is notable that asporin, of examples in which inactivation of individual genes of the involved proteins indicates that very little collagen is eliminated over the life of an individual; however, at the same time, fibers defective as a result of fatigue have to be repaired. It is possible that the very variable dimensions of the fibers in the interterritorial matrix result from adding newly synthesized collagen molecules at the fiber surface and thus gradually increasing the diameter to provide mechanical stability.

A number of molecules bound at the surface of collagen fibers are likely to prevent further accretion of collagen. It is plausible that these molecules will need to be removed before new collagen molecules can be added to a fiber for remodeling or repair.

It appears that even though the collagen itself does not turn over, molecules on the fiber’s surface are continuously removed and replenished. Molecules with putative roles in regulating fibril formation are found among those that can bind collagen in vitro.

Cartilage oligomeric matrix protein

Cartilage oligomeric matrix protein is a molecule primarily found in cartilage, where it is quite abundant at a concentration of around 0.1% of the tissue’s wet weight. The molecule is made up of five identical subunits, each with a molecular weight of around 87,000 Da. The five subunits are held together by a coiled-coiled domain close to the N-terminal end, and disulfide bridges further stabilize the binding. The subunits are made up of several modules, including some binding calcium. At the C-terminal end, there is a globular domain that is involved in interactions with other proteins in the matrix. The molecule can be viewed as a bouquet of tulips tied together at their stalks (see Fig. 2.2).

Cartilage oligomeric matrix protein, also referred to as thrombospondin-5, is a member of this family of proteins. Cartilage also contains other thrombospondins that share the same properties, with thrombospondin-1 and thrombospondin-4 being particularly abundant. These thrombospondins, however, contain an extension beyond the coiled-coil domain in the N-terminal that has a heparin-binding motif, in this manner adding additional interacting sites. Whereas thrombospondin-4 contains five identical subunits, thrombospondin-1 contains only three.

The three-dimensional structure of the C-terminal domain of thrombospondin-1 has been resolved, and its organization has been found to be stabilized by a large number of calcium ions. Because the C-terminal domain of the various thrombospondins shows a great deal of conservation, it is likely that its structure is similar in all five members of the family.

Cartilage oligomeric matrix protein has been shown to bind to collagens type I and II, where each individual C-terminal globular domain provides high affinity in the nanomolar range. Four binding sites are evenly distributed...
along the collagen molecule. There is one at each end and two positioned along the filament such that the distance is similar between the four binding sites. Even though each COMP molecule has five identical binding sites, an individual molecule can engage only one binding site on each collagen molecule and not span the distance between two such sites. Therefore, each COMP molecule has the potential to bind to four different collagen molecules. The quarter stagger arrangement of collagen in the fiber and the fact that a pentamer microfibril unit appears to exist may relate to the four similarly spaced collagen binding sites in the molecule. COMP accelerates and provides faster collagen fibril formation in vitro. It appears that this effect is mediated by the COMP molecule bringing together several collagen molecules to facilitate their interactions in the forming fiber. The COMP molecule does not remain bound directly to the surface of the forming fiber. The COMP molecule thus appears to function as a catalyst to enhance fibril formation.

In the cartilage of growing individuals and notably in growth plates, COMP is primarily localized close to the cells in the territorial matrix, where it may have a role in stimulating collagen fibril formation. Cartilage oligomeric matrix protein has the ability to interact with all four NC domains of collagen type IX with similar high affinity in the nanomolar range. The interaction is mediated via the C-terminal globular domains (see Fig. 2.2).

In adults, COMP is localized primarily in the interterritorial matrix and may be bound to collagen type IX or one of the matrixins, which in turn bind to the surface of the collagen fiber (see Fig. 2.2). The role of COMP in adult cartilage appears to be stabilization of the collagen fiber network. Mutations in the calcium-binding domain of COMP, as well as in the C-terminal domain, have been shown to lead to severe growth disturbances in the form of pseudochondrodysplasia or multiple epiphyseal dysplasia. A feature of these conditions is that material retained in the endoplasmic reticulum of chondrocytes contains both COMP and collagen type IX.

On the other hand, the COMP-null mouse shows no detectable alteration in phenotype. It is possible that other molecules compensate for the lack of COMP function in such mice. Carilage oligomeric matrix protein is significantly upregulated in early stages of OA, even long before the diagnosis of OA is apparent. At the same time, the protein already deposited is cleaved and released from the tissue. Indeed, assay for such fragments released into body fluids has been used to measure altered cartilage metabolism as a biomarker for arthritic disease.

Decorin
Decorin was the first molecule in the leucine-rich repeat (LRR) protein family to be cloned and sequenced. This family of molecules in the ECM contains four subclasses with a total of 12 members, all of which appear to share the function of binding to collagen (see Fig. e2.2). One functional domain of these molecules is a central LRR region, where residues, particularly leucine, are found at conserved locations in each repeat of some 25 amino acids, albeit somewhat variably long. Most of these molecules have 10 to 11 such repeats, and the entire domain contains a disulfide loop structure. One subclass of the LRR proteins contains modules with only six repeats.

For further details on decorin, see ExpertConsult.com. Decorin binds tightly to the fibril-forming collagens with a KD in the nanomolar range. Binding is close to the C-terminus of the collagen as shown for collagen type I. Binding occurs via the LRR region and particularly involves repeats 4 and 5. The critical sequence has been identified as SYRIADTNIT.

Via its binding to collagen, decorin inhibits the formation of collagen fibers in vitro in a dose-dependent manner. Accordingly, mice lacking decorin have irregular collagen fibers with increased collagen fibril diameter, particularly prominent in skin. They do not show increased early joint pathology, thus indicating that other molecules may compensate for the lack of decorin in articular cartilage.

Decorin is bound to collagen fibers in tissue, with the glycosaminoglycan chains being free to interact with other molecules. Thus, decorin can cross-bridge to neighboring collagen fibers, as well as to other molecules in the local environment (see Fig. 2.2).

Fibromodulin and lumican
Fibromodulin and lumican belong to the same subclass of LRR proteins but with a gene arrangement distinct from that of decorin. Other members of this subclass are keratan, osteoadherin, and PRELP. All these molecules except PRELP contain tyrosine sulfate residues in the N-terminal extension. Notably, the relative proportion of such sulfate residues is variable both with regard to the relative proportion of candidate tyrosine residues that are sulfated within a given molecule and with regard to the number of such tyrosine residues that may carry a sulfate. One extreme is represented by fibromodulin, which contains up to nine sulfate residues; osteoadherin contains up to eight, lumican up to four, and keratan only one. PRELP, in contrast, contains a cluster of basic residues that contribute heparin-binding activity for this molecule.

Collagen binding of fibromodulin has been studied extensively. It has been shown that the molecule inhibits fibril formation in vitro. Fibromodulin-null mice show altered collagen fibril dimensions, particularly apparent in the tail tendon. Unexpectedly in view of the inhibitory effects observed in vitro, the tendon contains a much larger number of thin fibrils. An explanation appears to be a higher abundance of the related lumican molecule, which could be shown to bind to the same site on the collagen, albeit with somewhat lower affinity. It thus appears that lumican may guide early events in fibril formation. The molecule may then be competed away by fibromodulin to induce a shift to the short rather than the long collagen fibril.

The molecule may thus have roles in regulating mineralization, which is relevant to the development of OA. Asporin, similar to decorin, biglycan, and fibromodulin, appears to bind transforming growth factor-β (TGF-β). There is a set of LRR proteins with only six repeats.
One variable of almost all the molecules in the family is an N-terminal extension of generally less than 20 amino acids, which may contain a variety of substituents (see Fig. e2.2). Some of the molecules also have a C-terminal extension. There is also variation in glycosylation of the repeat domain, which usually contains a few N-linked oligosaccharides. In some of the small LRR proteins, such as fibromodulin and lumican, some of the oligosaccharides may contain a variably long array of 6-O-sulfated lactosamine repeat disaccharides (see Fig. 2.3) extended to form the glycosaminoglycan keratan sulfate.

The three-dimensional structures of decorin and biglycan have been resolved by x-ray crystallography. These molecules contain one (decorin) or two (biglycan) glycosaminoglycan chains bound at the N-terminal extension. These chains are chondroitin sulfate or dermatan sulfate, depending on the tissue, though very similar between the molecules in a given tissue. In articular cartilage, the chain is a low-epimerized dermatan sulfate in which a few of the glucuronic acid residues have been epimerized to iduronic acid, thereby increasing its structural variability. This glycosaminoglycan has the capability of specific interactions with other molecules in the matrix, including binding other dermatan sulfate chains.

Based on the x-ray crystallographic data presented, the LRR family of molecules forms a curved structure in which two molecules form a dimer in the crystals that overlap in opposite directions in about 50% of their length such that the N-terminus of one molecule is located in the middle of the curved domain of the other. Support for such a dimeric structure was obtained by other techniques such as gel filtration with online dynamic light scattering. Electron microscopy indicates that decorin and biglycan, as well as chondroadherin, may also exist in a monomeric form at very low concentrations (Mörgelin and Heinegård, unpublished work).

The presence of the proteoglycan as a monomer or a dimer has specific relevance for interactions and functions. Thus, a monomeric molecule has only one of each interacting site, but a dimer may exhibit two of each binding site.

The function of the domain with clustered tyrosine sulfate residues is becoming clearer. The domains in fibromodulin and osteoadherin mimic heparin in many interactions and bind growth factors (e.g., fibroblast growth factor-2 [FGF-2]), cytokines (e.g., oncostatin M and IL-10), MMPs (e.g., MMP-13 for fibromodulin), and a number of matrix proteins via their heparin-binding domains (e.g., PRELP and chondroadherin). Thus, the fibromodulin on a collagen fiber appears to extend its tyrosine sulfate domain, which can bind to molecules with cationic domains. One such charged structure is the NC4 domain of collagen type IX, which has been shown to interact with the anionic N-terminal extension of fibromodulin, as schematically outlined in Fig. 2.2.

All the molecules in this family appear to bind to collagen via their LRR domain with dissociation constants ranging from 1 to 10 nM. Only in a few cases has it been established exactly where along the collagen that the LRR protein binds.

Such proteins include epiphycan, mimecan, and opticin. They all contain N-terminal extensions carrying glycosaminoglycan chains (epiphycan), tyrosine sulfate residues (mimecan), or O-glycosidically linked oligosaccharides (opticin) (see Fig. e2.2). Mimecan has also been named osteoglycin. There is limited knowledge on alterations of these proteins in joint disease. They do show interesting differences between different types of cartilage. Mimecan is present in articular cartilage, menisci, and intervertebral disks but virtually absent in nasal and tracheal cartilage, but epiphycan is prominent in the latter cartilage and absent from the others. Opticin is particularly prominent in connective tissues other than those of joints.

FIG. E2.2 Note the variability in the N-terminal domain between molecules, which shows negatively charged groups of different character (glycosaminoglycan, tyrosine sulfate, polyaspartate, or a cluster of basic amino acids). The C-terminal extensions of two members have distinct features. The dimeric form found for decorin and biglycan on x-ray crystallography is illustrated schematically. CHAD, Chondroadherin; CS, chondroitin sulfate; ECM, extracellular matrix; LRR, leucine-rich repeat; OSAD, osteoadherin.
Heparin and heparan sulfate
A glycosaminoglycan not prominent in cartilage is heparan sulfate, which has structural features overlapping those of heparin. Heparan sulfate is found as side chains of extracellular perlecan (discussed later), which has a very large protein core with several domains interacting with a number of other proteins in the ECM. The proteoglycan contains an N-terminal domain with some three glycosaminoglycan chains and a C-terminal domain with up to two chains. These chains may be heparan sulfate or chondroitin sulfate (discussed later).

Heparan sulfate contains two types of disaccharide repeats consisting of either a glucuronic acid and an N-acetylgalactosamine or an iduronic acid and an N-acetylgalactosamine. The hexosamine carries an O-sulfate group, and some of the residues have an additional N-sulfate instead of the N-acetyl group. Also, the uronic acid may be sulfated, usually in its 2 position. Thus, there is extensive variability in the building blocks, which are then assembled in variably long stretches with different repeat stretches of low- and high-sulfated residues.

Other heparan sulfate–containing proteoglycans are found at the cell surface of the chondrocyte. These include four different syndecans,50 which contain a domain intercalated in the cell membrane, and some six glypicans, which are joined via a glycosylphosphatidylinositol linkage.51 The syndecans contain an intracellular signaling domain, and as discussed later, they are involved in a number of cell reactions, and bound molecules can induce signal transduction.

FIBERS AND NETWORKS WITH COLLAGEN TYPE VI AS THE MAIN CONSTITUENT
There is a different fibrillar network in cartilage with a more restricted distribution in tissue. It has collagen type VI as a major constituent and is primarily present in the territorial matrix surrounding the cells (see Fig. 2.1).

Collagen type VI
Collagen type VI has a distinctive network of beaded filaments. This molecule contains three different α chains with a central triple-helical domain flanked by globular domains. The N-terminal portion, particularly the α1(VI) chain, contains nine von Willebrand factor A (vWFA)-like repeats. In addition, the C-terminal portions of all three chains contain two vWFA repeats, as well as other motifs with less clear functions. The vWFA domain is found in many proteins, where it is involved in protein–protein interactions.

While still within the cell, two collagen type VI molecules associate in an antiparallel fashion such that the dimer is flanked by the N-terminal domains, and the two C-terminal domains are placed so that they form two interior globular structures along the filament formed by the two triple helices. Two such dimers associate laterally to form a tetramer, with the globular domains at each end representing a pair of the vWFA-rich globules. Internally in the triple-helical central filament are found the two globular structures representing the C-terminals.52 The tetramer is secreted from the cell, and its N-terminal structures lay the ground for further associations with fibrils involving both end-to-end and side-to-side interactions. This assembly process appears to be governed by other molecules, particularly members of the LRR protein family.

Biglycan and decorin
Biglycan and decorin both bind with high affinity to the collagen type VI N-terminal domain, independently of their glycosaminoglycan side chains. This binding is a prerequisite for formation of the collagen type VI beaded filament network in vitro. Regulation of collagen type VI assembly also depends on the presence of the chondroitin–dermatan sulfate side chains, where the two present in biglycan provide more efficient filament formation than the single chain in decorin does. The two closely related proteoglycans appear to bind to the same site, which interestingly does not seem to involve the triple-helical domain in this collagen.53

Matrilins
Cartilage matrix protein (CMP matrilin-1) was the first of the four matrilins identified. These proteins (for references, see Klat and colleagues56) contain vWFA domains. Matrilin-1 has two such domains in each of the three identical subunits with a molecular mass of around 50,000 Da; matrilin-3, with four subunits, has only one such domain.

Matrilin-1 and matrilin-3 are quite restricted to cartilage and show similar distribution between different tissues; the others have more ubiquitous distribution. Interestingly, matrilin-1 is even further restricted and not found in articular cartilage and intervertebral disks, but it is particularly prominent in trabecular cartilage. The protein is present in the more immature cartilage of the femoral head during earlier phases of development and can be seen in the early bone anlagen. Its role in cartilage is becoming clearer, and data indicate roles in collagen network function and integrin binding (for references, see Klatt and colleagues). Its two vWFA homology domains (only one in matrilin-3) may mediate the ability of the protein to bind to collagen. Matrilin-1 was initially isolated because of its apparent ability to bind to aggregan. Mutations in matrilin-1 can induce multiple epiphyseal dysplasia or pseudoachondroplasia with skeletal malformations, similar to those caused by mutations in the interacting partners of the proteins, such as collagen IX.56

Matrilin-1 can be isolated from cartilage as a mixed polymer that contains subunits of matrilin-1, as well as matrilin-3. The functional significance of this heterocomplex is not understood.57

A module cross-linking to other matrix constituents
Decorin and biglycan appear to also have roles in the completed network. Collagen type VI isolated from chondrosarcoma cartilage–like tissue contains biglycan or decorin bound at the N-terminal globules. The proteoglycan in turn has a bound member of matrilin-1, -2, or -3. This interaction is tight with a dissociation constant on the order of nanomolar. The matrilin, in turn, binds to a procollagen type II molecule or a completed collagen fiber. Alternatively, matrilin can bind to an aggrecan molecule (see Fig. 2.2).54

Thus, collagen type VI seems to be a center in a scaffold that binds to the other molecules in the network. Collagen type VI is found only in the territorial matrix closer to cells and is absent from the interterritorial matrix at some distance from the cells. Information on alterations in collagen type VI in joint disease is limited, but it is important to note that the protein is found to be particularly enriched in tissue subjected to load.

MOLECULES INTERACTING AT THE CELL SURFACE AND MODULATING CHONDROCYTE BEHAVIOR
It is important to realize that chondrocytes have the ability to both degrade the matrix and replenish lost molecules with new constituents in a process of remodeling the tissue in response to material fatigue or to altered load. The cells are guided in this endeavor by receptors at their surface that recognize specific molecules in the matrix (see Fig. 2.2). Binding elicits specific signals that will either induce cellular spreading and migration by engaging the cytoskeleton or lead to alterations in transcription and protein synthesis. Other stimuli that affect cells include mechanical forces, which provide signals that cross-talk with those from other interactions at the cell surface. Indeed, some data indicate that some of the signals elicited by mechanical load involve integrins.58

INTEGRIN BINDING PROTEINS
The integrins contain an α chain noncovalently bound to a β chain. The cells in connective tissues contain either a β1 or a β3 chain in conjunction with one of many α chains. The various integrins have different preferred ECM ligand proteins.59 As an example of the organization of integrins, members of one such family with four members bind to collagen. They contain a β1 chain in combination with an α5, α6, or α1 chain. These various integrins have different tissue distributions such that the one with α6 appears to be unique to cartilage, but the others have more ubiquitous distributions. Their binding to collagen may elicit different responses and result primarily in either altered production of enzymes degrading the matrix or production of building blocks such as collagen molecules.

A number of factors limit our ability to discern which integrins are present on chondrocytes in tissue. One factor is limited accessibility by the antibodies used, and another is changes in the presence of integrin during the long procedure for cell isolation. Thus, the current information on the presence of integrins at the cell surface is variable. It is likely that a number of integrins change their expression on chondrocytes in normal and in pathologic tissue in response to environmental factors.

Many of the molecules binding to integrins are not unique for cartilage. The collagen-binding integrins do not appear to be specific for a particular fibril-forming molecule. One exception is chondroadherin. This protein appears to be restricted to cartilage and can bind αβ integrin but not other integrins containing the same β chain.

Chondroadherin
Chondroadherin is a member of the LRR protein family that is in its own subclass. This protein differs from other LRR family members in that the C-terminal cysteine loop is double, and the protein has a short C-terminal extension of basic amino acids. The protein has no N-terminal extension.
Chondroadherin specifically binds to cell-surface $\alpha_\beta$, integrin with a sequence in one of its C-terminal disulfide loops. Interestingly, integrin binding elicits signals that do not induce cell spreading. The very C-terminal short extension peptide of chondroadherin contains a different cell-binding sequence that engages the heparan sulfate chains of cell-surface proteoglycans, notably syndecans. Binding leads to cell spreading, as well as enhanced integrin activity. The combined engagement of integrins and heparan sulfate leads to cells forming focal adhesion complexes.

Chondroadherin is present in articular cartilage but virtually absent from menisci. The protein is particularly enriched during growth in the prehypertrophic zone of the growth plate where cell multiplication is slowed, in line with its in vitro effects on cell behavior. Interestingly, the protein appears to be lost early in the process of joint damage in OA (unpublished observations).

**Fibronectin**

Fibronectin is present in most tissues and can actually form its own fibrils, which appear to have roles in guiding matrix assembly and cell migration. The protein contains two identical subunits held together by disulfide bonds close to their C-terminal end.

Fibronectin contains a collagen-binding domain with preference for denatured collagen (gelatin). There are integrin-binding domains in which the RGD (arginine–glycine–aspartic acid) sequence represents the classic motif of integrin binding. This motif in fibronectin preferentially binds $\alpha_\beta$, integrin, although $\alpha_\beta$, integrin can also interact. Fibronectin also has other integrin-binding domains.

Another motif is represented by the two heparin-binding domains on each subunit, each about 20 kDa. These domains can interact with heparin sulfate proteoglycans at the cell surface, including syndecan, which also represent signaling molecules.

Although the details are not known, fragments of fibronectin (e.g., those containing either of the heparin-binding domains), when added to cartilage in explant culture, as well as injected into the joint, will stimulate chondrocytes to produce proteases and induce cartilage breakdown. In contrast, the intact fibronectin molecule will not have such effects on cells. It is possible that fragmentation of fibronectin is one mechanism for propagating joint destruction in disease. The active fragments have not yet been identified in body fluids.

Fibronectin is already upregulated in articular cartilage at very early stages of OA in many species, including humans. The functional consequences of this upregulation are not known but may represent part of an attempt at repair. A result is further availability of fibronectin, which may be fragmented to further increase tissue degradation.

**Collagen and the Discoidin Domain 2–Containing Receptor**

Collagen has an additional cell receptor for discoidin domain receptor 2 (DDR-2). This receptor has been shown to be upregulated in mice in which OA is developing. DDR-2 will bind collagen type II, thereby inducing upregulation of MMP-13 production. Exposure of collagen type II may be enhanced by removal of proteins bound at the fibrillar surface, thus making the molecule available for interactions.

**Hyaluronan**

Hyaluronan is an extremely long glycosaminoglycan chain that is distinct from all the others in that it is not bound to a protein core. It does not contain any sulfate groups. The backbone is made up of one to several thousand repeating disaccharides of glucuronic acid and N-acetylglosaminic. As discussed earlier, hyaluronan specifically binds to a domain in aggrecan that is essential for the formation of aggregates.

Hyaluronan also interacts with specific cell-surface receptors. These receptors are CD44 molecules that interact with a minimally long hexasaccharide sequence of the polymer. The interactions with hyaluronan are important for organizing the pericellular environment and providing signals to the cell. CD44 occurs in many different splice forms with variable presence on different cells, but a role for such variability in joint disease is unclear. Another receptor for hyaluronan is RHAMM, also a signaling receptor and, similar to CD44, it is present on a variety of cells.

**Ligands for Heparan Sulfate/Syndecans**

In addition to the heparin-binding domains present in fibronectin, various other matrix proteins contain such domains. Examples are most members of the thrombospondin family, with the exception of COMP, many MMPs, CILP, and PRELP. As discussed earlier, isolated heparin-binding domain fragments can have variable effects on, for example, cells, depending on their fine structure. Effects range from inducing cartilage breakdown in vitro and in vivo to decreasing bone breakdown by targeting osteoclast precursors.

Interestingly, as discussed earlier, the N-terminal tyrosine sulfate domain of fibromodulin can mimic heparin in many interactions and bind other proteins to crossbridge networks, as well as sequester growth factors.

**Other Molecular Functions**

In joint disease, inflammation is a frequent component causing pain and limiting function. The inflammation is usually chronic, and one issue is whether components from cartilage can propagate the inflammatory activity. It is a long-standing observation that when cartilage is removed in joint arthroplasty, the inflammation recedes, thus indicating that factors released from the tissue have a role in propagating inflammation.

It has been shown that some patients with RA have circulating antibodies to collagen type II. Furthermore, an arthritis condition can be elicited by injecting mice and rats with collagen type II (collagen-induced arthritis). The disease can be transferred by antibodies to specific collagen type II epitopes. It is hence possible that antibody binding activates complement and inflammation.

More recent findings indicate that matrix proteins may activate innate immunity. Fibromodulin has been shown to be active as immune complexes in activating the classical pathway of complement, although it does not bind to the same site. Fibromodulin binds to the head domains of C1q rather than to the triple-helical stalk region with ensuing deposition and activation of C4 and C3.

Interestingly, fibromodulin can also recruit factor H by binding to a different site than that for C1q and thereby inhibit further activation of the complement cascade. Whether different fragments released in disease will have different roles in complement activation remains to be shown.

In other experiments, it has been demonstrated that biglycan can also bind the triple-helical stalk of the complement factor C1q and in doing so inhibit activation of the classical pathway of complement. It appears that other LRR proteins such as biglycan and decorin can also bind factors involved in regulating the complement cascade. One example is a tight interaction with C4BP having a role in regulating complement activity.

Cartilage oligomeric matrix protein is a potent activator of the alternative pathway via its C-terminal globular domain. Interestingly, this activity can be observed in synovial fluid from subjects with both OA and RA, as well as in blood in the form of COMP-C3b complexes created between the activator COMP and the C3b fragment formed.

**Binding and Sequestering Growth Factors in the Extracellular Matrix**

The ECM of cartilage contains a number of factors that are sequestered and thereby bind to specific interaction partners. On degradation of the matrix, one can predict that these factors are released and affect cellular activities. In particular, a number of growth factors have been found to have the capacity to bind to matrix proteins.

**Transforming growth factor-β**

Transforming growth factor-β has been shown to bind to a number of matrix proteins, and binding to some members of the LRR protein family has been investigated in particular. All three TGF-β variants bind to bacterially expressed decorin, biglycan, and fibromodulin, as well as to these proteins with the full range of posttranslational modifications. More recently, there are indications that asporin can also bind TGF-β. It has been demonstrated that active TGF-β can be released from decorin or treatment with MMP-3. Indeed, cartilage contains substantial amounts of TGF-β, which has been extracted and purified from the tissue.

**Fibroblast growth factor**

Some matrix proteins contain heparan sulfate side chains, which are likely to bind growth factors within the FGF family. In the case of syndecan at the cell surface, these side chains appear to be involved in presenting the growth factor to its receptor.

**Other Molecules in the Extracellular Matrix**

Perlec was found to have a central role in cartilage development when a mouse with the gene inactivated was developed. Most of these mice die during early development as a result of problems with the heart and major
blood vessels, but those that survive until birth show major growth disturbances with an extensively altered growth plate lacking a large proportion of the collagen fibers. Further studies have revealed that chondroitin sulfate side chains specific for perlecan can actually accelerate and catalyze collagen fiber formation. GP-39 is a protein upregulated in OA. It shares homology with a chitinase, but the true activity of the protein is not known. GP-39 is additionally expressed in a number of other tissues, particularly in disease.

Cartilage matrix also contains a number of proteins that are made elsewhere and are normally found primarily in the circulation. There is a preference for certain proteins, and low-molecular-weight basic proteins (e.g., lysozyme) in particular appear to bind to the matrix. Whether these molecules contribute specific functions is not known.

**FRAGMENTS OF MATRIX PROTEINS RELEASED DURING CARTILAGE BREAKDOWN AS MOLECULAR INDICATORS OF DISEASE**

In processes resulting in destruction of cartilage tissue, proteolytic enzymes degrade ECM proteins. Some of the fragments formed will no longer be retained in the tissue but are released to surrounding body fluids. By using sensitive immunoassays, such fragments can be quantified in synovial fluid or serum. This so-called molecular marker technology (biomarkers) is intended to provide new means of assaying ongoing active processes in articular cartilage. With further development, such techniques may be used in diagnosis, for estimation of the activity of the tissue-destroying process, to document effects of therapeutic intervention, and, most importantly, to discover processes during the early stage before clinical symptoms become apparent. One example is the demonstration that COMP can be used to identify patients who will have the most extensive joint destruction in both OA and RA.5 6 7 8 9 Also, a number of collagen fragments created by cleavage with collagenases, as well as by subsequent gelatinase activity, have been used to monitor disease. With further development of the technology, we hope to be able to identify indicators specific to a particular pathologic process in a given tissue. Thus, it should be possible to identify the activity of a process in the meniscus on the one hand and activity in cartilage on the other.

As discussed earlier, some of the fragments released will activate immune responses with the potential to further enhance disease activity.

**REPAIR AND REGENERATION OF CARTILAGE**

Cartilage ECM molecules have extremely long half-lives, and the cells of cartilage are not renewed. In addition, it is difficult to imagine how newly synthesized molecules would fully and functionally integrate into the cartilage matrix. However, anecdotally, it is believed that some individuals can repair cartilage to a limited extent, and there are animal models that demonstrate genetic differences in the ability to repair cartilage. Although the genetic differences are not known for sure, some growth factors involved in development of cartilage appear to play a role, such as the Wnt family, or TGF-B.

When cartilage is damaged, a number of repair strategies have been developed, including (1) replacing bad cartilage with good cartilage from the same joint, (2) using patient chondrocytes to make new ECM, (3) attempting to recruit or implant patient stem cells, and (4) other strategies that attempt to replace the cartilage matrix with an artificial matrix. We will briefly review recent advances in these technologies.

**REPLACING CARTILAGE**

Intact cartilage from the same joint is used to replace damaged cartilage in surgical techniques including mosaicplasty or surgery using morcelized cartilage. The primary surgical technique is called osteochondral autologous transplantation (OAT) mosaicplasty. This technique involves an open or arthroscopic transplantation of multiple cylindrical osteochondral grafts from the relatively less weight-bearing periphery of the articular surface to the cartilage defect, thus providing a hyaline cartilage–covered resurfacing. OAT mosaicplasty has demonstrated acceptable long-term clinical outcomes, given the appropriate indication for surgery, with a limitation being the defect size.

In another surgical technique, osteochondral allograft transplantation is also used. If the cartilage defect is too large for an autograft or if a patient has failed a cartilage repair procedure, then a fresh osteochondral allograft (OCA) is a single-stage technique for large osteochondral defects, particularly in a setting of extensive subchondral bone loss. Chondrocyte viability seems to be very important in this technique, and fresh OCAs stored at physiologic temperature have the highest level of chondrocyte viability. A study of 60 patients with femoral condylar allografts showed 95% graft survival rate at 5 years and 85% at 10 years.

**MICROFRACTURE AND AUTOLOGOUS CHONDROCYTE IMPLANTATION**

This has become a substantial research area in the field of cartilage repair. Two surgical techniques are used, microfracture (MF) of the subchondral bone to recruit bone marrow mesenchymal stem cells to populate the cartilage defect and a related technique, autologous chondrocyte implantation (ACI), a two-part procedure in which chondrocytes are isolated from intact non-weight-bearing regions of the joint, expanded and seeded differentiated in the laboratory and reimplanted into the osteochondral defect in the weight-bearing region. The MF technique is considered the first-line treatment given its minimally invasive nature, technical ease, limited surgical morbidity, and relatively low costs. In a study with 7-year follow-up, 80% of patients rated themselves as improved with patients younger than 35 years showing the most improvement. Biopsies after MF have noted that approximately 10% had hyaline cartilage, with the majority having predominantly fibrocartilage. In general, patients demonstrated clear improvement in knee function at 24 months after MF but inconclusive durability and treatment failure beyond 5 years.

Autologous matrix–induced chondrogenesis combines MF surgery with the application of a bilayer collagen membrane that physically stabilized the clot and may guide and enhance marrow-derived repair. In a study using a BST-CarGel (Piramal Like Sciences, Bio-Orthopaedic Division), treatment with ACI or MF resulted in greater lesion filling and superior repair tissue quality compared with MF treatment alone; however, clinical symptoms were equivalent between groups.

Autologous chondrocyte implantation is approved for use in the United States and is most useful for younger patients who have single defects larger than 2 cm. Disadvantages include the need for two-stage procedures and an open arthrotomy, expense, and a significant rate of reoperation for graft hypoplasia, specifically with first-generation ACI treatments. Second- (porcine membrane) and third-generation (matrix-associated) ACI treatments show promise but are not approved in the United States at this time. In a series of more than 200 patients treated with ACI for larger lesions, ACI provided durable outcomes with a survivorship of 71% at 10 years and improved function in 75% of patients. Magnetic resonance imaging findings confirmed complete defect filling in half of patients at final follow-up.

**STEM CELLS**

The limited expansion capacity of de-differentiated chondrocytes and their increasing inefficiency at redifferentiation during extended culture has led to the use of mesenchymal stem cells (MSCs) for autologous cartilage repair of larger defects. MSCs are found in numerous human tissues, including bone marrow, articular cartilage, and synovial membrane. Cell-based therapy is becoming an established element of modern health care and is predicted to grow as knowledge and implementation of cell biology, biomaterials, and regenerative medicine increases. MSCs are defined as adherent self-renewing, fibroblastoid-like cells that can differentiate into osteoblasts, adipocytes, and chondrocytes in vitro. These characteristics are present in the MSCs derived from the bone marrow, adipose tissue, and synovial joint tissues. These cells can be differentiated in vitro to chondrocytes and used as chondrocytes or can be injected into the joint, where they may differentiate into chondrocytes or, more likely, provide factors and immune inhibitors that facilitate joint repair. Bone marrow MSCs were found to be at least as effective as chondrocytes for articular cartilage repair in improving symptoms of patients. However, chondrogenically induced bone marrow MSCs hold the inherent risk of either forming transparent fibrocartilaginous repair tissue or undergoing terminal differentiation to form calcified cartilage, subchondral bone overgrowth, or intraskeletal osteophyte formation. Thus, alternatives to bone marrow MSCs are sought.

Adipose-derived MSCs, now called adipose-derived stem cells (ASCs), offer the advantage that they are abundant in adipose tissue or liposuction samples. Two recent reviews summarize efforts to fabricate or regenerate articular cartilage using scaffolds and growth factors with ASCs. Cells from the synovium, synovial fluid, periosteum, the infrapatellar fat pad, trabecular bone, and muscle have also been used to differentiate chondrocytes, but no definitive positive regeneration has been documented. There is the possibility of recruiting these native cells from the tissues to the cartilage defect, but this has not yet been productive. The area of cell-based strategies for cartilage repair is thus under intensive study. However, the field has not
moved as fast as was predicted, primarily because of safety, cost, efficacy, and regulatory hurdles.

TISSUE-ENGINEERED CARTILAGE

A variety of combinations of MSCs with various scaffolds using biomaterials approaches, bioreactors, and combinations of cartilage- and bone-inducing materials have been developed. For a review, please see Boushell et al.

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REFERENCES

Bone structure and function

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INTRODUCTION

Bone is a complex natural composite material that undergoes millions of loading cycles during a lifetime without failure. Its structure is hierarchical, being organized differently at the organ (whole bone), tissue (material), and molecular (collagen–mineral) levels.

The mechanical functions of bone are the most widely recognized and are often described in terms of strength and stiffness. Although strength and stiffness are important, bone is particularly effective at dissipating energy that could cause a fracture over repeated cycles of loading. Beyond its mechanical function, the marrow cavity and the porous trabecular bone in the ends of long bones and in the vertebrae and iliac crest are regions in which red blood cells are formed (red marrow). In osteoporosis, the differentiation of cells in the bone lineage can be partly diverted to adipocytes, and the marrow becomes more fatty (yellow marrow). Because cancellous bone has a large surface area in contact with vascular marrow, it is ideal for the long-term exchange of calcium ions. In osteopenia, regions of cancellous bone are affected first. This is why the vertebral column, which is mainly composed of cancellous bone, is affected earlier and more severely in osteoporosis than even the femoral neck and hip.

MICROSCOPIC (TISSUE) LEVEL

At the microstructural level, bone is organized differently for different functions (Fig. 3.2). In humans, bone tissue can be divided into three broad categories based partly on the arrangement of the collagen fibers and partly on whether it has replaced preexisting bone: (1) woven bone, (2) primary bone, and (3) secondary bone.

Woven bone is characterized by randomly oriented collagen fibers, which tend to be smaller in diameter than those in more highly organized primary or secondary bone. Woven bone is not lamellar, is very porous and may become highly mineralized. Woven bone can be deposited de novo without any bony or cartilaginous substrate or anlage, but it can also be formed as part of the process of endochondral ossification, either at the growth plate during development or during fracture repair. Woven bone proliferates rapidly because it has a large cell-volume ratio, which makes its role in fracture repair ideal. It is often found associated with osteosarcoma, probably because of proliferation especially of the cellular periosteum. The function of woven bone is primarily mechanical, rapidly providing both temporary strength and scaffolding upon which lamellar bone may be deposited. However, it can also be associated with pathologic processes that involve inflammatory cytokines.

Primary bone must be deposited on a preexisting substrate and is organized into lamellar layers. Because of this, trabecular plates, which are composed mainly of primary lamellae, cannot be replaced after they are perforated. This accounts for the loss of trabeculae with age and is part of the reason that it is difficult to reverse osteoporotic changes after trabecular connectivity has been lost. Primary lamellar bone also forms in rings around the endocortical and periosteal surfaces of whole bone (circumferential lamellae). Primary lamellar bone itself is not very vascular and can become very dense. Primary bone can also be arranged in concentric rings around a central canal—much like a secondary haversian system, except without a definable cement line. These primary osteons tend to be small with few concentric lamellae. In reality, primary osteons are modified vascular channels that have “filled in” by the addition of lamellae to the surface of the vascular space.

Secondary bone is the product of the resorption of preexisting bone and its replacement with new bone. This can occur within dense cortical bone (resulting in a secondary osteon, or haversian system) or begin on the surfaces of trabeculae (sometimes called a hemi-osteon). The distinction between primary and secondary bone is important because it is likely that control of primary bone apposition is different from replacement of preexisting bone by secondary bone. A secondary haversian system has a central vascular canal 50 to 90 µm in diameter. The blood vessels in the canal have the characteristics of capillaries and are generally paired within the canal. Venous sinuoids and lymphatic vessels are not found in haversian canals, although it has been suggested that lymphatic vessels may exist.

The vessel walls...
contain no smooth muscle but are fenestrated capillaries lined by an incomplete layer of endothelial cells, similar to vessels in other blood-forming organs like the spleen and bone marrow. The vessel is accompanied by about 60% of the time by two to seven unmyelinated or poorly myelinated nerve fibers. Because the capillaries have no smooth muscle, these nerves do not serve a vasomotor function but are primary sensory nerves or autonomic fibers from the sympathetic nervous system. Both primary afferents and sympathetic nerve fibers express neuropeptides that may play some role in regulating bone remodeling. Afferent nerve fibers also express GAP43, which is associated with axonal growth, and may reflect the need for vessel growth and reinnervation during bone remodeling.

Lining cells (resting osteoblasts) cover the haversian canal, which is surrounded by a series of concentric lamellae containing bone cells—osteocytes. The relationship between the size of the osteon and the size of its canal is a measure of the balance between bone resorption (osteon diameter) and bone formation (canal diameter). The secondary osteon is bounded by a cement line, probably composed largely of sulfated glycosaminoglycans. The cement line forms an effective boundary that can arrest cracks in bone and stop them from growing to a critical size.

**COMPOSITION OF BONE**

Bone is composed of organic and mineralized components, mainly consisting of a matrix of cross-linked type I collagen mineralized with nanocrystalline, carbonated apatite. Bone matrix incorporates a small fraction of noncollagenous proteins that serve to control collagen assembly and size as well as the process of mineralization and cell attachment. The mineral comprises about 65% to 67% of the bone by weight, the organic component about 22% to 25%, and water the remaining fraction (~10%). Within the organic fraction, type I collagen makes up about 90%, with the remainder accounted for by several minor collagen (types III and V) and a variety of noncollagenous proteins, most of them extracellular, with cell protein accounting for about 15% of the noncollagenous proteins in bone.

Type I collagen in bone is formed by a triple helix composed of two α1 chains and a single α2 chain. At either end of the collagen molecule are an N-terminal peptide and a C-telopeptide, which can be cleaved when bone is resorbed and which are used to measure bone resorption biochemically. These individual triple helical collagen molecules self-associate into a periodic arrangement of parallel molecules spaced in quarter-staggered array at distances of 40 nm between their ends to form collagen fibrils (Fig. e3.1). The holes plus the overlap zones give the collagen its banded appearance with a D-periodic spacing of 67 nm on average. The spaces between the ends of the collagen molecules are known as hole zones, and the 35-nm gap zones that run longitudinally parallel between the molecules are known as pores. Hydroxyapatite \([\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]\), which has small and poorly formed crystals, nucleates within these spaces. However, most of the mineral in bone, about 73%, is in the form of more highly carbonated apatite that forms plates about 300 nm long that lie outside the collagen fibril (extrafibrillar). The initial deposition of mineral—primary mineralization—occurs rapidly after new bone is deposited, with about two thirds of the eventual mineral content achieved within about 3 weeks. As the bone tissue matures, the mineral crystals grow, become more platelike, and orient themselves parallel to one another and to the collagen fibrils. This process of crystal growth is sometimes called secondary mineralization and occurs over the next year or so until full mineralization is achieved. In cancellous bone, the c-axis or long axis of the mineral crystal aligns with the longitudinal axis of the trabeculae; in cortical bone, the mineral orients to the long axis of the osteon. Other elements can easily substitute into the hydroxyapatite crystal, changing its character and its ability to withstand mechanical loads. Collagen fibers can become cross-linked through the action of enzymes, which results in immature or reducible

**FIG. 3.1** Bone consists of the diaphysis (shaft), the metaphysis, and the epiphysis. In growing children, the growth (epiphyseal) plate separates the epiphysis from the metaphysis. During growth, the periosteal surface of the metaphysis must be constantly resorbed, while concurrent bone formation occurs on the endocortical surface to convert the thin-walled but flared metaphysis into the narrower but thicker-walled diaphysis (McNeel tetrachrome stain).

**FIG. 3.2** The three general microscopic types of bone, defined morphologically. Woven bone is composed of disorganized, randomly oriented collagen fibers. It is found at sites of fracture or inflammation. Lamellar bone can be divided into primary and secondary. Primary bone is deposited in layers by direct apposition on a substrate. It is found circling the endocortical and periosteal circumferences of a long bone and within trabeculae. Secondary bone is formed by replacement of primary bone through the process of resorption and subsequent new bone formation. (Woven bone: polarized light. Primary lamellar bone: left, basic fuchsin; right, polarized light. Secondary osteonal bone: left, toluidine blue; right, scanning electron microscopic image.)
COLLAGEN

Hole zones

Pore zones

Periodic staggered structure
Mineral associated with gaps in stagger

"Young Bone":
Cross-links associated with N and C end terminals of collagen
Enzymatic cross-links

"Old Bone":
Non-end terminal associated cross-links
Nonenzymatic cross-links

FIG. E3.1 In the collagen fibril, molecules are organized in a quarter-staggered array, with hole and pore zones between them where mineral can deposit. The hole zones give the collagen fibril its banded appearance when viewed by electron microscopy. Cross-links are formed either through the action of enzymes (divalent and trivalent cross-links) or through nonenzymatic processes, the latter forming advanced glycation end products that can make bone behave in brittle fashion.
(divalent) cross-links that mature through the action of lysyl oxidase to irreducible (trivalent) cross-links such as pyridinoline and deoxypyridinoline. Because divalent cross-links are rapidly converted into mature trivalent cross-links under normal circumstances, the quantity of divalent cross-links in bone is an indirect measure of remodeling rate. Divalent and trivalent cross-links are associated with the N- and C-terminals of the collagen molecule. Cross-links can also be formed between the fibers without the action of enzymes. These cross-links are formed by the condensation of arginine, lysine, and free sugars or through various oxidation reactions, which results in the formation of advanced glycation end products. Accumulation of advanced glycation end products occurs in diabetes, making the bone more brittle and increasing the risk of fracture.

The skeletal noncollagenous proteins comprise 10% to 15% of total bone protein. These molecules constitute a wide range of polypeptide species and have roles in multiple skeletal processes in addition to functioning as structural scaffolding within the bone matrix. One important feature of bone that has been largely overlooked until recently is water, which comprises about 10% of bone volume and can be found in either free-flowing or bound forms. Water stabilizes collagen structure but also allows sliding at the interface between the mineral and the collagen, which increases the ductility and toughness of bone. Because proteoglycans are hydrophilic, they may be important in retaining the water in some compartments.

PROTEOGLYCANS

Proteoglycans (PGs) are a ubiquitous family of molecules composed of a core protein and one or several covalently attached sulfated glycosaminoglycan chains. The glycosaminoglycans are linear polymers of repeated disaccharide units of hexosamine and hexuronic acid, except for keratan sulfate, in which hexuronic acid is replaced by galactose. The core proteins attached to the glycosaminoglycans are a diverse protein group and range in size from 10 to 500 kDa. The wide variety of protein structure may aid in directing the unique functional roles of each PG family.

The bone matrix contains PG families of several primary structures, including the following:

1. Hyaluronan/CD44, chondroitin sulfate–containing PGs are expressed in several regions of bone. Hyaluronan is expressed in focal regions within periosteum and endosteum and surrounding most of the major bone cell types including osteoblasts, osteoprogenitor cells, osteoclasts, and osteocyte lacunae. CD44 is a cell-surface hyaluronan receptor that may play roles in guiding bone development and has been localized to osteoclasts, osteocytes, and bone marrow cells. Versican, a chondroitin sulfate–containing PG, is often enriched during bone formation and may provide a temporary framework in newly formed cartilage matrix during bone development. Results from cultured mesenchymal cells derived from a mouse model carrying a disrupted versican gene (the hdf transgenic mouse) also suggest that mature versican PG may play a role in directing limb chondrocyte aggregation depending on the surrounding extracellular matrix (ECM).

2. Heparan sulfate PGs (HSPGs) are produced by osteoblast and osteoclast lineage cells. These molecules play important roles in cell–cell interactions during bone formation by trapping autocrine and paracrine heparin-binding fibroblast growth factor (FGF) family members, as well as acting as coreceptors with the FGF receptors. Also, other secreted molecules bind HSPGs, such as transforming growth factor-β (TGF-β, betaglycan) and osteoprotegerin (OPG) (syndecans). The bioactivity of these factors is modulated by HSPGs, potentially through focusing of concentrations of these potent molecules near differentiating cells.

3. Small leucine-rich PGs are the most abundant of the PGs in bone matrix and include decorin, biglycan, fibromodulin, lumican, and osteoadherin. These molecules help to provide the structural organization of the bone matrix and interact with specific growth factors and collagen to increase factor concentration and bioactivity in the matrix (Fig. 3.3). The localization of these proteins in mature bone varies; whereas decorin may localize with specific matrix areas, biglycan is evenly distributed throughout the matrix. Whereas decorin-null animals have alterations in collagen fibril size and bone shape,13 biglycan-null animals demonstrate reduced bone mass because of lower osteoblast numbers and also show reduced numbers of osteoclasts. Small leucine-rich PGs play an essential role in the regulation of growth factor activity. In this regard, decorin, biglycan, and fibromodulin all possess the ability to bind to TGF-β; however, decorin is the best characterized of these proteins for the ability to bind this factor. Decorin enhances TGF-β binding with its cognate receptors and enhances its bioactivity and, in concert, may act to sequester TGF-β in the collagen fibrils, thus reducing its activity. TGF-β activity is associated with increased apoptosis of osteoprogenitors; therefore, biglycan and decorin appear to be essential for maintaining mature osteoblast numbers through regulation of the proliferation and survival of bone marrow progenitor cells.

OSTEOCALCIN

Osteocalcin (OC) is a polypeptide posttranslationally modified to carry dicarboxylic glutamyl (Gla) residues, which relies on vitamin K for proper production (another identifier for OC is bone γ-carboxyglutamic acid [Gla] protein [BGLAP or BGP]). In humans, vitamin K is primarily a cofactor in the enzymatic reaction that converts glutamate residues into γ-carboxyglutamate residues in these vitamin K-dependent proteins including OC but also in proteins involved in blood clotting such as factor IX. These Gla-containing motifs are thought to enhance calcium binding, which may function to control mineral deposition and bone remodeling. A nine-residue domain proximal to the N-terminal of secreted OC shares high homology with the

FIG. 3.3 Proteoglycans (PGs) regulate growth factors at several levels in the bone matrix and in bone cells. These polypeptides trap and locally concentrate endocrine and paracrine growth factors within the matrix. Soluble PGs such as decorin, biglycan, and perlecan also modulate activity through binding, thus controlling the concentrations of bioavailable factor. Membrane-bound PGs such as betaglycan and syndecan modulate ligand–receptor interactions and thus play roles in regulating intracellular signaling. FGF, Fibroblast growth factor; FGFR, fibroblast growth factor receptor; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand; TGF-β, transforming growth factor-β; TGF-βRI/RRII, transforming growth factor-β receptor types I and II. (Adapted from Lamoureux F, Baud’huin M, Duplomb L, et al. Proteoglycans: key partners in bone cell biology. Bioessays 2007,29:758-71.)
corresponding regions in known propeptides of the γ-carboxyglutamic acid–containing blood coagulation factors. This common structural feature may be involved in the posttranslational targeting of these proteins for γ-carboxylation.

Osteocalcins may also act as a hormone to regulate the activity of osteoclasts and their precursors. In support of this, the skeleton of the OC-null animal manifests osteopetrosis compared with wild-type litter mates. In humans, OC is expressed largely by osteoblasts and osteocytes, and the measurement of this protein in serum has been used as a marker of bone turnover. OC messenger RNA (mRNA) is upregulated by vitamin D through interactions with trans-acting factors in vitamin D response elements in the OC promoter.23 Because of its cell-specific expression, the OC promoter has proven to be invaluable as an active, functional DNA to drive foreign complementary DNAs in osteoblasts in transgenic animals.

OSTEOPONTIN

Osteopontin (OPN), also referred to as secreted phosphoprotein-1, is a member of the SIBLING (small integral-binding ligand N-linked glycoprotein) family, which is a group of noncollagenous ECM proteins involved in bone mineralization. The genes coding for these proteins are localized to human chromosome band 4q21-25; have similar exon arrangements; and include those coding for dentin matrix protein-1, dentin sialoprotein, dentin phosphoprotein, integrin-binding sialoprotein, and matrix extracellular phosphoglycoprotein (MEPE). The SIBLING proteins share common structural features, such as multiple phosphorylation sites, a highly acidic nature, the presence of an arginine–glycine–aspartic acid cell attachment domain, and proteolysis-resistant acidic serine aspartate–rich MEPE-associated motif. OPN binds tightly to hydroxyapatite and may be involved in the anchoring of osteoclasts to the mineral of bone matrix. The vitronectin receptor, which has specificity for OPN, is focused within the osteoclast plasma membrane in the regions involved in the binding process. Long bones from OPN-null mice are indistinguishable from those from wild-type litter mates by radiography, but the relative amount of mineral in the more mature areas of the bone (central cortical bone) of the OPN-null mice is significantly increased, as is the mineral maturity (mineral crystal size and perfection) throughout all regions of the bone.24 In vitro, exogenous OPN inhibits inorganic pyrophosphate (PPi)-dependent mineralization of a cultured osteoblast cell line.25 These findings indicate that OPN is a potent inhibitor of mineral formation as well as crystal growth and proliferation.

OSTEONECTIN

Osteonectin, also referred to as secreted protein acidic and rich in cysteine (SPARC), is a phosphoprotein that is the most abundant noncollagenous polypeptide expressed in bone. The mature protein is selectively secreted by osteoblasts; it binds hydroxyapatite, collagen fibrils, and vitronectin at distinct sites and may allow proper organization of the bone matrix through contacts with the cellular surface. Osteonectin also inhibits cellular proliferation through arrest of cells in the G1 phase of the cell cycle. It may regulate the activity of platelet-derived growth factor, vascular endothelial growth factor, and FGF-2. The osteonectin crystal structure has revealed a novel follistatin-like component and an extracellular calcium-binding region containing two EF-hand motifs. Osteonectin-null mice develop severe osteopenia, which indicates that this gene may have roles in osteoblast proliferation and in mineralization.

ALKALINE PHOSPHATASES AND ECTONUCLEOTIDE PYROPHOSPHATASE AND PHOSPHODIESTERASES

Alkaline phosphatases are widely distributed and are membrane-bound glycoproteins that hydrolyze monophosphate esters.26 The liver–bone–kidney alkaline phosphatase, referred to as tissue-nonspecific alkaline phosphatase (encoded by the ALPL gene), acts as a lipid-anchored phosphoethanolamine and pyridoxal 5′-phosphate ectophosphatase. Loss-of-function mutations in the ALPL gene lead to hypophosphatasia, which is characterized by marked defects in bone mineralization and is lethal in the infantile form. The PPi produced by cells inhibits mineralization by binding to crystals, and the presence of PPi may thus prevent the soft tissues from undergoing mineralization. The degradation of PPi, to inorganic phosphate by ALPL in bones and teeth may facilitate crystal growth; therefore, it is thought that loss of function of the ALPL gene in hypophosphatasia results in accumulation of PPi, and reduced skeletal mineralization. Ectonuclease pyrophosphatase/
autosomal dominant hypophosphatemic rickets (ADHR), a metabolic bone disorder of isolated renal phosphate wasting. Full-length FGF-23 (32 kDa) is the biologically active form of the protein and is inactivated upon cleavage into 20- and 12-kDa protein fragments. Intracellular cleavage of FGF-23 occurs between residues R179 and S180 via the SPC furin (PCSK3). The importance of this event is underscored by the fact that patients with GALNT3 inactivating mutations do not efficiently produce bioactive FGF-23.21 Thus providing a molecular interaction that controls the ability of osteoblasts and osteocytes to regulate serum phosphate concentrations and potentially bone mineralization (see Fig. 3.4). FGF-23 acts in the kidney to inhibit phosphate reabsorption by reducing expression of the proximal tubule type 1 sodium–phosphate cotransporters Npt2a and Npt2c. The subsequent low phosphate level results in marked osteomalaeria and rickets, fracture, and dental anomalies. Application of in situ hybridization to adult trabecular bone revealed the presence of FGF-23 mRNA in osteocytes and flattened bone-lining cells. In regions of active bone formation, newly formed osteocytes and osteoprogenitor cells also express FGF-23.22 FGF-23 levels are elevated in vivo by increased serum phosphate and 1,25-dihydroxyvitamin D concentrations, and FGF-23 then competes with the feedback loop by reducing phosphate reabsorption and 1,25-dihydroxyvitamin D production in the kidney. Evidence also indicates that FGF-23 is directly regulated by parathyroid hormone (PTH) in osteocytes.26 Whether FGF-23 has direct effects on the skeleton is uncertain because the FGF-23 coreceptor α-Klotho is predominantly expressed in the kidney and parathyroid glands. However, because FGF-23 is produced in bone, FGF-23 expression and its actions on serum phosphate concentrations may be coordinated with intraskeletal signals to allow proper bone formation and mineralization.

PHEX

X-linked hypophosphatemia, a disorder of rickets and osteomalacia, is caused by inactivating mutations in PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome). PHEX encodes a protein that is similar to the M 13 family of metallopeptidases. The subsequent low phosphate level results in marked osteomalaeria and rickets, fracture, and dental anomalies. Application of in situ hybridization to adult trabecular bone revealed the presence of FGF-23 mRNA in osteocytes and flattened bone-lining cells. In regions of active bone formation, newly formed osteocytes and osteoprogenitor cells also express FGF-23.22 FGF-23 levels are elevated in vivo by increased serum phosphate and 1,25-dihydroxyvitamin D concentrations, and FGF-23 then competes with the feedback loop by reducing phosphate reabsorption and 1,25-dihydroxyvitamin D production in the kidney. Evidence also indicates that FGF-23 is directly regulated by parathyroid hormone (PTH) in osteocytes.26 Whether FGF-23 has direct effects on the skeleton is uncertain because the FGF-23 coreceptor α-Klotho is predominantly expressed in the kidney and parathyroid glands. However, because FGF-23 is produced in bone, FGF-23 expression and its actions on serum phosphate concentrations may be coordinated with intraskeletal signals to allow proper bone formation and mineralization.

Dentin matrix protein-1

Dentin matrix protein-1 (DMP1), similar to OPN, is a member of the SIBLING gene family. DMP1 is highly expressed in osteocytes and is composed of 513 residues but is secreted in bone and dentin as 37-kDa N-terminal (residues 17–253) and 57-kDa C-terminal (residues 254–513) fragments from a 94-kDa full-length precursor (see Fig. 3.4). Recombinant DMP1 binds calcium-phosphate ions and the N-telopeptide region of type I collagen with high affinities, so in vivo DMP1 may regulate local mineralization processes in bone and teeth. The C-terminal portion of DMP1 has been implicated in DNA binding, in gene regulation, and as an integrin-binding protein. Inactivating mutations in DMP1 result in the metabolic bone disease autosomal recessive hypophosphatemic rickets, which is associated with elevated FGF-23 levels in these patients. As shown in the Dmp1-null mouse (and in the Hyp mouse model of X-linked hypophosphatemia), the primary cellular defect caused by loss of Dmp1 may be an alteration in osteoblast to osteocyte maturation, leading to inappropriate differentiation of osteoblasts or early osteocytes that are incapable of binding to one or several of four FGF receptors (FGFRs). FGFRs normally exist as an inactivated monomer. With FGF binding in the presence of heparin/heparan sulfate, the FGFRs dimerize, which leads to the autophosphorylation of tyrosine residues. The FGFR family has potent effects on bone development. This is clearly evident by the fact that activating mutations in FGFR1 and FGFR2 are responsible for disorders of craniosynostosis and limb patterning, and FGFR1 and FGFR3 mutations result in disorders of hypochondroplasia and achondroplasia. The FGFRs interact with HSPGs and are sequestered within the mineralized matrix. In addition, the HSPG syndecan may stabilize FGF-FGFR interactions and promote FGF signaling and bioactivity.

Growth factors

Multiple growth factors, either produced within bone or circulating to bone, are critical for skeletal development and function. These factors may be sequenced with the bloodstream or may be produced by the major bone cell types and act as paracrine and autocrine factors.

Insulin-like growth factors

The insulin-like growth factors IGF-1 (somatomedin C) and IGF-2 (somatomedin A) are produced primarily in the liver but are also produced in bone. These factors predominately circulate complexed with IGF binding proteins (IGFBPs) to facilitate their transport to tissues. IGFBPs can either enhance or inhibit IGF activity. IGF-1 and IGF-2 act through the IGF-1 receptors (IGFRI and IGFIR2) and possess bioactivity that promotes cell proliferation and differentiation.

The IGF-1-null mouse has reduced cortical bone and femur length; however, trabecular density is increased. In vitro findings suggest that IGF-1 also increases osteoclastogenesis, and IGF-1-null mice have reduced levels of receptor activator of nuclear factor-κB ligand (RANKL) in osteoblasts isolated from the femoral bone. These findings indicate that MEPE likely has a role as an important gene for the negative regulation of skeletal mineralization.

Bone morphogenetic protein family

Bone morphogenetic proteins (BMPs) are members of the TGF-β superfamily. There are now more than 20 BMP-related proteins, which are classified into subgroups based on structure and function. These factors play important roles in diverse aspects of bone development, including differentiation of mesenchymal cells into osteoblasts through differentiation of these precursor cells into cells of the osteoblastic lineage, and by inhibiting their differentiation into myoblastic lineage cells. BMPs also increase osteoclastogenesis, which is tightly coordinated with osteoblastogenesis. BMPs activate specific receptors and induce cell signaling by phosphorylating cytoplasmic receptor–regulated Smads, which enter the nucleus to recruit transcription factors and enhance gene expression. The human disorder fibrodysplasia ossificans progressiva is a disease of dramatic ectopic bone formation, which can be accelerated after blunt trauma. A recurrent mutation in activin receptor IA/activin-like kinase 2, a BMP type I receptor, was reported as the molecular cause of fibrodysplasia ossificans progressiva.29 These findings underscore the potent effects of BMP signaling on bone formation.
of osteoblasts enhances the binding of Runx2 to the Cbfa1 consensus sequence in the OC promoter and may therefore have a role in differentiation.

**Transforming growth factor-β**

Transforming growth factor-β (TGF-β) has several functions in many cell types. TGF-β1, TGF-β2, and TGF-β3 all function through the type I and type II TGF-β receptors. The type I TGF-β receptor forms a heterodimer with the type II TGF-β receptor. TGF-β stimulation leads to activation of SMAD2 and SMAD3, which form complexes with SMAD4 that accumulate in the nucleus and regulate the transcription of target genes.

Transforming growth factor-β is the most abundant growth factor in human bone; it is localized within the bone matrix and has functions both during embryonic development and in mature bone. During embryonic development, TGF-β1 plays a role in cell migration, controlling epithelial–mesenchymal interactions, and the formation of cellular condensations, which influence bone shape. This factor also plays a key role in inducing mesenchymal cell differentiation to either chondrocytes or osteoblasts. In adult bone, TGF-β1 controls osteoblast differentiation, which affects matrix formation and mineralization. TGF-β1 inhibits the expression of the differentiation markers Runx2 and OC in osteoblast cell lines, and its functions interplay with those of other osteogenic factors, such as the PTH and Wnt/β-catenin systems.

The skeletal disorder Camurati-Engelmann disease (CED) highlights the importance of TGF-β1 in skeletal formation. CED is a progressive diaphyseal dysplasia characterized by hyperostosis and sclerosis of the diaphyses of the long bones. The TGFβ1 gene was screened and three different heterozygous missense mutations were found in exon 4 in the nine families examined. All of the mutations in the CED patients were located either at C225 or near R218, which suggests the importance of this region in activating TGF-β1 in the bone matrix.

**Platelet-derived growth factor and vascular endothelial growth factor**

All platelet-derived growth factors (PDGFs) and vascular endothelial growth factors (VEGFs) are dimers of disulfide-linked polypeptide chains, encoded by nine different genes that direct production of four different PDGF chains (PDGF-A, PDGF-B, PDGF-C, and PDGF-D) and five different VEGF chains (VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor). All members of these families carry a growth factor core domain that is necessary for receptor activation. PDGFs mediate their bioactivity through two receptors, PDGFR-α and PDGFR-β. These receptors both have five extracellular immunoglobulin loops for ligand binding and an intracellular tyrosine kinase domain. The VEGFs act through a homologous family of receptors, VEGFR1, VEGFR2, and VEGFR3. PDGFs act primarily as paracrine growth factors.

Platelet-derived growth factor is chemotactic and mitogenic for osteoblasts and osteoprogenitor cells, and it upregulates cytokines that are crucial to bone healing. This factor also destabilizes blood vessels during healing to allow sprouting of new vessels. VEGF is produced by many cell types including fibroblasts, hypertrophic chondrocytes, and osteoblasts. VEGF may act not only in bone angiogenesis and vascular differentiation but also in aspects of development, such as chondrocyte and osteoblast differentiation, as well as osteoclast recruitment.

**BONE CELLS**

Bone development and the adaptation of the adult skeleton to mechanical needs and hormonal changes depend on the ability of bone cells to resorb and form bone in the right places and at the right time. Bone growth, modeling, and remodeling are defined by the spatial and temporal relationship between bone resorption and bone formation. Osteoclasts resorb bone, osteoblasts form bone, and osteocytes detect the need for bone augmentation or reduction and coordinate the activity of osteoclasts and osteoblasts.

**OSTEOCLASTS**

Osteoclasts are the primary bone-resorptive cells. They are needed for bone modeling, which leads to changes in the shape of bones, and for bone remodeling, which maintains the integrity of the adult skeleton. Osteoclasts originate from precursors of the monocyte/macrophage family of the hematopoietic lineage that differentiate to multinucleated cells upon stimulation with RANKL and macrophage colony-stimulating factor (M-CSF) (Fig. 3.5). Upon completing bone resorption, all osteoclasts undergo programmed cell death or apoptosis and disappear from the bone surface.

**Osteoclast morphology and function**

Osteoclasts adhere firmly to bone through the interactions established between integrins expressed in the osteoclast membranes with collagen, fibronectin, and other bone matrix proteins. Expression of αv and δv integrin is induced during osteoclast differentiation, and the integrin binds to the amino acid sequence Arg-Gly-Asp present in OPN and bone sialoprotein. The importance of these events for osteoclast activity is underscored by the inhibition of resorption with competitive Arg-Gly-Asp ligands and a progressive increase in bone mass caused by osteoclast dysfunction in mice null for β3 integrin.

The intimate contact between the osteoclast and the bone matrix creates a space called the sealing zone. There is also polarization of the osteoclast fibrillar actin into a circular structure called the actin ring, containing podosomes composed of an actin core surrounded by αvβ3 integrins and associated cytoskeletal and signaling proteins. Thus, the area in which the osteoclast apposes the bone is isolated from the general extracellular space and becomes acidified by the activity of a proton pump and a chloride channel. The low pH in this area dissolves the mineral and exposes the organic matrix, which is subsequently degraded by the activity of lysosomal cathepsin K and matrix metalloproteases. These degrading enzymes are

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**Fig. 3.5** Osteoclast differentiation is governed by receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) secreted by osteoblasts and osteocytes, which control various steps of the osteoclast differentiation process, including precursor proliferation, commitment, differentiation, and maturation. Osteoprotegerin (OPG), which is also secreted by osteoblasts and osteocytes, acts as a decoy receptor for RANKL and reduces osteoclast differentiation. (From Bellido T, Plotkin LI, Brузанни A. Bone cells. In: Bellido T, Plotkin LI, editors. Basic and applied bone biology. San Diego: Academic Press; 2014, p. 27-46.)
transported into acidified vesicles that fuse with the osteoclast plasmalemma, forming a villous structure referred to as the ruffled border. This structure and the actin ring are essential features of a resorbing osteoclast, and abnormalities of either structure lead to arrested bone resorption.

The cytoplasmic domains of integrins serve as platforms for signaling pathways involved in osteoclast function, such as the kinase Src, which is crucial for osteoclast attachment and resorption. Src regulates podosome disassembly and ruffled membrane formation by its ability to interact with the focal adhesion–related kinase Pyk2 and the proto-oncogene c-Cbl. Rho, Rac, and guanine nucleotide exchange factor Vav3, which activates guanosine diphosphate exchange to guanosine triphosphatases, also play a central role in modulating the resorptive capacity of osteoclasts by modulating the actin cytoskeleton. Osteoclast resorption is initiated through the cytosol to the basolateral surface and discharged to the extracellular milieu or directly released to the surrounding fluid after osteoclast retraction from the resorption pits.

Osteoclast formation and differentiation
Mature, multinucleated osteoclasts are formed by fusion of mononuclear precursors of the monocyte/macrophage lineage (see Fig. 3.5). The earliest recognized osteoclast precursor is the granulocyte-macrophage colony-forming unit, which also gives rise to granulocytes and monocytes. Osteoclast precursors proliferate in response to growth factors such as interleukin-3 (IL-3) and colony-stimulating factors like granulocyte-macrophage colony-stimulating factor (GM-CSF) and M-CSF to form postmitotic, committed mononuclear osteoclast precursors, which differentiate and fuse to form multinucleated osteoclasts under the influence of RANKL, a member of the tumor necrosis factor (TNF) family of ligands.

M-CSF and RANKL are critical for osteoclastogenesis, and deletion of M-CSF, RANKL, or RANK (the receptor for RANKL expressed by osteoclasts and their precursors) inhibits osteoclast differentiation, leading to osteopetrosis in mice. Both M-CSF and RANKL are expressed by bone marrow stromal cells and osteoblastic cells, as well as T lymphocytes and other cell types in pathologic settings. Importantly, osteocyes have now been found to be a major source of M-CSF and RANKL, as well as OPG, the RANKL decoy receptor, and deletion of RANKL from osteocytes leads to osteopetrosis, demonstrating a central role of osteocytes in osteoclastogenesis (see Fig. 3.5). Whereas M-CSF contributes to osteoclast differentiation, migration, and survival by binding to its receptor c-Fms on osteoclast precursors, RANKL facilitates osteoclast formation via direct binding to the receptor RANK. RANKL expressed on the cell surface and is also secreted as a soluble form. Although the soluble form of RANKL is found in the circulation and its presence is sufficient to induce differentiation of osteoclast precursors in vitro, its actual role in osteoclast formation in vivo remains unproven.

RANKL expression is upregulated by hormones and cytokines known to induce osteoclastogenesis. This explains the long-observed property of primary osteoblastic cells or osteoblastic cell lines that, upon treatment with vitamin D, PTH, or IL-11, IL-6, TNF, and IL-1, support osteoclast development when co-cultured with osteoclast precursors derived from spleen or bone marrow. RANKL mediates several aspects of osteoclast differentiation, including fusion of mononuclear precursors into multinucleated cells, acquisition of osteoclast-specific markers, attachment of osteoclasts to the bone surfaces, stimulation of resorption, and promotion of osteoclast survival. Although M-CSF contributes to RANKL effects, RANKL appears to play a dominant role in bone resorption. Thus, whereas M-CSF–null mice recover with time from the decreased osteoclast number and activity, RANKL knockout mice do not. Furthermore, RANKL appears to stimulate osteoclast formation and resorption in mice even in the absence of functional M-CSF.

RANKL activates several signal transduction pathways involving the recruitment of the adapter protein TRAF6 (TNF receptor–associated factor 6) to the intracellular domain of the receptor RANK. TRAF6 in turn activates kinase-dependent signaling as well as transcription factors. Among them, NF-κB has been shown to be involved in osteoclastogenesis, including fusion and calcification, leading to upregulation of c-Fos. c-Fos, in turn, binds to nuclear factor of activated T cells, c-Fos; NF-κB subunits have normal survival rates, and inhibition of NF-κB activation via a dominant-negative IKK2 does not affect the ability of IL-1 to promote osteoclast survival. Therefore, the relevance of NF-κB signaling for osteoclast survival is still controversial.

Osteoclast apoptosis
All osteoclasts undergo apoptosis and disappear from the bone surface after completing bone resorption. High concentrations of extracellular calcium, similar to the ones present in resorption cavities, induce osteoclast apoptosis in vitro and may be the triggering event. Fas ligand stimulates osteoclast apoptosis, and Fas-deficient mice exhibit more osteoclasts and decreases in bone mass, which suggests that this pathway controls osteoclast life span in vivo. Osteoclast apoptosis might also result from loss of survival signals provided by integrin interactions with the matrix or by changes in the production of cytokines or growth factors that preserve osteoclast viability. Potential antiapoptotic factors are M-CSF and RANKL, the same cytokines that induce osteoclast differentiation. TNF-α and IL-1 also delay osteoclast apoptosis. Antiapoptotic factor Bcl-2 has been shown to undergo nuclear translocation, leading to upregulation of IL-1 to promote osteoclast survival. Therefore, the relevance of NF-κB signaling for osteoclast survival is still controversial.

Regulation of osteoclast generation and survival
In the bone-remodeling unit, whereas the rate of osteoclast generation determines the extension of the bone-remodeling unit, the life span of osteoclasts determines the depth of resorption. Although both genesis and apoptosis of osteoclasts lead to changes in osteoclast number and bone resorption, alteration of osteoclast life span might represent a more effective mechanism to accomplish rapid changes in bone resorption rate.

Sex steroids have profound effects on osteoclasts. Both estrogens and androgens inhibit osteoclast generation by regulating the production of pro-osteoclastogenic cytokines (e.g., IL-6 and IL-1) by cells of the stromal/osteoblastic lineage. Estrogens also induce apoptosis of mature osteoclasts. This, together with an inhibitory effect of the hormones on osteoelastic generation, leads to a decrease in the rate of bone remodeling.

Mice receiving excess glucocorticoids exhibit reduced osteoclast progenitors, but cancellous osteoclast number does not decrease in the early phases of the disease because glucocorticoids prolong the life spans of preexisting osteoclasts. This effect may account for the early transient increase in bone resorption in patients with hyperglucocorticoidism. In contrast to the rapid prosurvival effect of glucocorticoids on mature osteoclasts, glucocorticoids instead increase osteoclast apoptosis by reduction in the pool of osteoblastic cells that support osteoclastogenesis. This effect leads to the typical low remodeling rate observed in chronic glucocorticoid-induced osteoporosis.

OSTEOBLASTS
Osteoblasts are the cells responsible for bone formation. They originate from mesenchymal progenitors, which also give rise to chondrocytes, muscle cells, and adipocytes (Fig. 3.6). Commitment of mesenchymal cells to the osteoblastic lineage depends on the specific activation of transcription factors induced by morphogenetic and developmental proteins that carry out the functions of bone matrix protein secretion and bone mineralization. Upon completion of bone matrix formation, some mature osteoblasts remain entrapped in bone as osteocytes, some flatten to cover quiescent bone surfaces as bone-lining cells, and most die by apoptosis.

Osteoblast function
The main function of osteoblasts is to synthesize collagen type I and other specialized matrix proteins that serve as a template for the subsequent mineral deposition in the form of hydroxyapatite. Mature osteoblasts actively engaged in this process are recognized by their location on the bone surface and by their morphologic features typical of cells secreting high levels of proteins, cuboidal shape with large nucleus, enlarged Golgi apparatus, and extensive endoplasmic reticulum. Osteoblasts express high levels of alkaline phosphatase and OC, and the level of these proteins in blood reflects the rate of bone formation.
Interaction of osteoblasts among themselves, with lining cells, and with bone marrow cells is established by adhesion junctions, tight junctions, and gap junctions. Adhesion junctions mainly mediated by cadherins and tight junctions serve to join cells and facilitate their anchorage to the ECM. Changes in the expression level of the major cadherins expressed in osteoblasts, N-cadherin and cadherin 11, influence osteoblast differentiation and survival. Intercellular communication among osteoblasts and neighboring cells is maintained by cell coupling via gap junctions. Opening of gap junction channels contributes to coupling and the coordination of responses within a cell population. The major gap junction protein expressed in bone cells is connexin 43. Its absence or dysfunction leads to impaired osteoblast differentiation, premature apoptosis of osteoblasts and osteocytes, and deficient response to hormones and pharmacotherapeutic agents. Furthermore, gap junction communication is fundamental for the maintenance of a continuum from bone, where osteocytes reside, through bone surface cells, osteoblasts and osteoclasts, bone marrow cells, and endothelial cells of the blood vessels. This functional syncytium might be responsible for the coordinated response of the bone tissue to changes in physical and chemical stimuli, as will be discussed later. Interactions between osteoblasts and the bone matrix via integrins also modulate osteoblast differentiation, function, and survival. In particular, loss of antiapoptotic signals provided by the ECM causes apoptosis, a phenomenon referred to as anoikis.

**Osteoblast formation and differentiation**

The process of osteoblastogenesis can be divided into steps comprising proliferation, ECM development and mineralization, and apoptosis. Each stage is characterized by activation of specific transcription factors and genes leading to a succession of osteoblast phenotypic markers (see Fig. 3.6). Transcription factors of the helix-loop-helix family (Id, Twist, and Dermo) are expressed in proliferating osteoblast progenitors and are responsible for maintaining the osteoprogenitor population by inhibiting the expression of genes that characterize the osteoblast mature phenotype. Transcription factors of the activating protein family, such as c-fos, c-jun, and junD, are expressed during proliferation as well as later in the differentiation pathway and may activate or repress transcription. Runx2 and osterix are essential for establishing the osteoblast phenotype. Their absence from the mouse genome results in lack of skeletal mineralization and perinatal lethality. Runx2 and osterix regulate the expression of other genes that control bone formation and remodeling, including OC and RANKL. Runx2 regulates differentiation, survival, and function of osteoblasts by affecting several signaling pathways, including those activated by Wnts, BMPs, integrins, and the PTH receptor.

**Osteoblast apoptosis**

Upon completing the process of bone formation, 60% to 70% of osteoblasts die by apoptosis; the rest become lining cells or osteocytes. Apoptosis occurs throughout all stages of osteoblast life. The prevalence of osteoblast apoptosis in bone sections can be quantified by measuring fragmented DNA. Apoptosis of cultured osteoblasts has been extensively studied using several methods, including increased activity of initiator or effector caspases, the presence of cleaved genomic DNA by TUNEL or ISEL assay, and nuclear fragmentation and chromatin condensation using fluorescent dyes that bind to DNA. Examination of the nuclear morphology of cells transfected with fluorescent proteins containing a nuclear localization sequence has proven a particularly useful tool for studying apoptosis in cells co-transfected with genes of interest. Cell detachment from the substrate, changes in the composition of the plasma membrane, and changes revealing cell shrinkage are also features that have been used to detect and quantify apoptotic cells.

**Regulation of osteoblast generation and apoptosis**

Most major regulators of skeletal homeostasis influence both generation and survival of osteoblasts. The BMP and Wnt signaling pathways promote osteoblast differentiation, but whereas BMPs induce osteoblast apoptosis, Wnts inhibit it. BMPs induce apoptosis of mature osteoblasts as well as of mesenchymal osteoblast progenitors in interdigital tissues during the development of the hands and feet. Wnt signaling has a profound effect on bone as shown by the high-bone-mass phenotype of mice and humans with activating mutations of low-density lipoprotein receptor–related protein 5 (LRP5), which together with Frizzled proteins are receptors for Wnt ligands. Wnts stimulate differentiation of undifferentiated mesenchymal cells toward the osteoblastic lineage and stimulate differentiation of preosteoblasts. Canonical Wnt signaling in osteoblasts also affects osteoclasts by enhancing the expression of the RANKL decoy receptor OPG, which leads to inhibition of osteoclast development. In addition, Wnt signaling inhibits apoptosis of mature osteoblasts and osteocytes. The increased bone formation exhibited by mice lacking the Wnt antagonist known as secreted Frizzled related protein-1 (sFRP-1) is associated with decreased osteoblast and osteocyte apoptosis. The prevalence of osteoblast apoptosis is also decreased in mice expressing the high bone mass–activating mutation of LRP5 (G171V), which exhibit reduced ability to bind the Wnt antagonist sclerostin secreted by osteocytes. Consistent with this, sclerostin induces osteoblast apoptosis in vitro. Moreover, reduction of sclerostin levels by PTH and mechanical loading increases osteoblast number and activity as a result of stimulation of osteoblast differentiation and increased survival. Activation of Wnt signaling in vitro by ligands known to activate the so-called canonical as well as noncanonical pathways also prevents apoptosis of osteoblast progenitors and differentiated osteoblasts through a mechanism that involves the Src/ERK and PI3/AKT pro-survival kinases.

Glucocorticoids induce rapid bone loss resulting from a transient increase in resorption caused by delayed osteoclast apoptosis. This initial phase is followed by a sustained and profound reduction in bone formation and turnover caused by decreased osteoblast and osteoclast generation and increased osteoblast apoptosis.

Both persistent excess of PTH, as in hyperparathyroidism, and intermittent elevation of PTH (by daily injections) increase the number of osteoblasts. Sustained PTH elevation inhibits the expression of sclerostin, with a consequent increase in Wnt signaling and in differentiation of osteoblast precursors. A major effect of intermittent elevation of PTH is inhibition of apoptosis of osteoblasts, which thereby prolongs their life span and ability to form bone.

**OSTEOCYTES**

Osteocytes are former osteoblasts that become entombed during the process of bone deposition and are regularly distributed throughout the mineralized
bone matrix. Osteocyte bodies are individually encased in lacunae and exhibit cytoplasmic dendritic processes that run along narrow canaliculi excavated in the mineralized matrix. Osteocytes communicate with each other, with cells on the bone surface, and with cells of the bone marrow through gap junctions established between cytoplasmic processes of neighboring cells. Today it is accepted that osteocytes are the mechanosensory cells. Osteoblasts and osteoclasts are present on bone only transiently, in low number, and in variable locations. On the other hand, osteocytes are the most abundant resident cells and are present in the entire bone volume. Osteocytes are also the core of a functional syncytium that extends from the mineralized bone matrix to the bone surface and the bone marrow and all the way to the blood vessels. This strategic location permits the diffusion of molecules by mechanical signals as well as in levels of cell signaling and cell trafficking which allows amplification of the signals leading to adaptive responses.

**Osteocyte apoptosis: consequences and regulation**

Osteocytes are long-lived cells. However, similar to osteoblasts and osteoclasts, osteocytes die by apoptosis, and decreased osteocyte viability accompanies the bone fragility syndromes that characterize glucocorticoid excess, estrogen withdrawal, and mechanical disuse. Conversely, preservation of osteocyte viability might explain at least part of the antifracture effects of bisphosphonates, these findings raise the possibility that preservation of osteocyte viability by mechanical stimuli may be an important mediator of bone protection.

**Preservation of osteocyte viability by mechanical stimuli**

Osteocytes interact with the ECM in the pericellular space through discrete sites in their membrane, which are specialized integrins, as well as through transverse elements that tether osteocytes to the canalicular wall. Loading of the bones induces ECM deformation and fluid flow through the canaliculi, producing tension in the tethering elements and strain on osteocyte membranes. The consequent integrin engagement leads to intracellular signaling. Physiologic levels of mechanical strain imparted by stretching or pulsatile fluid flow prevent apoptosis of cultured osteocytes. Mechanotransduction is accomplished by molecular complexes assembled at caveolae and domains of the plasma membrane and composed of integrins, cytoskeletal proteins, and kinases, including the focal adhesion kinase FAK and Src, which results in activation of the ERK pathway and osteocyte survival. Intriguingly, a ligand-independent function of the estrogen receptor is indispensable for mechanically induced ERK activation in both osteoblasts and osteocytes. This observation is consistent with reports that mice lacking estrogen receptor-α and estrogen receptor-β exhibit a poor osteogenic response to loading.

In vivo mechanical forces also regulate osteocyte life span. Apoptotic osteocytes are found in unloaded bones or in bones exposed to high levels of mechanical strain. In both cases, increased osteocyte apoptosis is observed before any evidence of increased osteoclast resorption. Apoptotic osteocytes accumulate in areas subsequently removed by osteoclasts. Targeted ablation of osteocytes in transiently loaded bone results in more rapid activation of the Src/Shc/ERK signaling pathway through nongenotropic actions of the classical receptors for sex steroids. This effect requires only the ligand-binding domain of the receptor, and unlike the classical genotropic action of the receptor protein, it is eliminated by nuclear targeting.

Effects of glucocorticoid activity in bone may also contribute to induction of osteocyte (and osteoblast) apoptosis because aged mice exhibit higher serum levels of corticosterone, elevated adrenal weight, and increased expression in bone of 1β-hydroxysteroid dehydrogenase type 1 (1β-HSD1), the enzyme that amplifies glucocorticoid action. The apoptotic effect of glucocorticoids is reproduced in cultured osteocytes and osteoclasts in a manner strictly dependent on the glucocorticoid receptor. Induction of osteocyte and osteoblast apoptosis by glucocorticoids might result from the direct action of the steroids, because overexpression of the enzyme that inactivates glucocorticoids, 1β-HSD2, specifically in these cells abolishes the increase in apoptosis. Strikingly, in the osteocytic MLO-Y4 cell line, the proapoptotic effect of glucocorticoids is preceded by cell detachment caused by interference with FAK-mediated survival signaling generated by integrins. In this mechanism, Pyk2 (a member of the FAK family) becomes phosphorylated and subsequently activates proapoptotic JNK signaling. The proapoptotic actions of glucocorticoids may involve suppression of the synthesis of locally produced antiapoptotic factors, including IGF-1- and IL-6-type cytokines, as well as matrix metalloproteins, and stimulation of the proapoptotic Wnt antagonist SFRP-1.

**Preservation of osteocyte viability via nongenotropic actions**

Preservation of osteocyte viability by mechanical stimuli may be an important mediator of bone protection.

**Regulation of bone formation by osteocytes: sclerostin**

Osteocytes express sclerostin, the product of the Sost gene, which antagonizes several members of the BMP family of proteins and also binds to LRPs/LRP5, preventing canonical Wnt signaling. Loss of Sost in humans causes the high-bone-mass disorders van Buchem syndrome and sclerostosis. In addition, administration of an antisclerostin antibody increases bone formation and restores the bone lost after ovariectomy. Conversely, transgenic mice overexpressing Sost exhibit low bone mass. These lines of evidence demonstrate that sclerostin derived from osteocytes exerts a negative feedback control at the earliest step of the process by downregulating cell differentiation toward the osteoblast lineage. Moreover, PTH and mechanical loading downregulate the expression of sclerostin in osteocytes, which reveals a novel mechanism of bone anabolism triggered by osteocytes.

**Osteocytes as mediators of the anabolic actions of canonical Wnt signaling in bone**

Bone anabolic stimuli activate Wnt signaling, and human mutations of components along this pathway underscore its crucial role in bone accrual and maintenance. However, the cell responsible for orchestrating Wnt anabolic actions has remained elusive because genetic activation or deletion of components of the pathway in osteoblasts or their precursors only affect bone resorption without evident effects on bone formation. A recent study demonstrates that activation of canonical Wnt signaling exclusively in osteocytes increases (not decreases) resorption and induces bone anabolism, leading to high bone remodeling with bone gain. This effect is due to the fact that activation of the pathway in osteocytes not only decreases OPG as in osteoblasts but also increases RANKL, leading to a higher RANKL-to-OPG ratio that favors resorption, and in this setting, Wnt signaling also favors osteoblast–osteocyte differentiation. Thus, these findings demonstrate disparate outcomes of β-catenin activation in osteocytes versus osteoblasts and identify osteocytes as central target cells of the anabolic actions of canonical Wnt/β-catenin signaling in bone.

**Regulation of bone resorption by osteocytes: RANKL and osteoprotegerin**

The cues that signal bone resorption are not completely understood. Apoptotic osteocytes could regulate the recruitment of osteoclast precursors and their differentiation in two ways. Osteocyte apoptosis may indirectly stimulate osteoclastogenesis by inducing stromal/osteoblastic cells to secrete RANKL. In vitro, purified osteocytes produce RANKL, and treatment with decortin (Dex) or parathyroid hormone (PTH) increases RANKL expression. These findings strongly suggest that osteocytes are the major source of RANKL in vivo. Osteocytes also secrete OPG, which, as in osteoblasts, is regulated by the Wnt/β-catenin pathway. Mice lacking β-catenin in osteocytes are osteoporoic because increased osteoclast number leads to bone loss. However, in vivo, protein production is normal. Emerging evidence also points to osteocytes as an additional source of secreted M-CSF in bone. Together, these new findings suggest that osteocytes control the bone-remodeling process by regulating osteoclast and osteoblast differentiation and function.
Osteocytes sense the need for bone resorption and send signals to lining cells, which retract from the bone surface to allow the formation of a canopy under which remodeling occurs, called the bone-remodeling compartment (BRC). Osteoclast precursors are transported to the BRC by marrow capillaries, differentiate to mature osteoclasts under the influence of pro-osteoclastogenic and antiosteoclastogenic cytokines (receptor activator of nuclear factor-κB ligand [RANKL], macrophage colony-stimulating factor [M-CSF], and osteoprotegerin [OPG]) derived from osteocytes, and initiate bone remodeling. Osteoclast precursors from the bone marrow or the circulation differentiate into mature, bone-synthesizing cells in response to factors released from the bone matrix by resorption. Differentiation and function of osteoclasts are controlled by molecules derived from osteocytes, including sclerostin and Dkk-1. (From Bellido T, Plotkin LI, Bruzzaniti A. Bone cells. In: Burr DB, Allen MR, editors. Basic and applied bone biology. San Diego: Academic Press, 2014, p. 27-46.)

**Table 3.1**

<table>
<thead>
<tr>
<th>Process</th>
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<th>Morphology</th>
<th>Function</th>
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<td>Woven, lamellar</td>
<td>Increased mass</td>
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<td>A-F</td>
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<td>A-R-F</td>
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<td>(osteons, semi-osteons)</td>
<td>Repair of microdamage</td>
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<td>Repair</td>
<td>F</td>
<td>Woven</td>
<td>Prevention of bone loss</td>
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<td>Rapid mechanical</td>
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* A, Activation; F, formation; R, resorption.

**Osteocytes and the bone-remodeling compartment**

Lining cells play an important function in initiating bone remodeling by retracting from quiescent bone surfaces and allowing the formation of a canopy over osteoclasts and osteoblasts in the bone multicellular unit. On the endocortical surface, this canopy presumably encases bone marrow osteoblast precursors and is penetrated by blood vessels that provide hema-topoietic osteoclast progenitors. The canopy, associated capillaries, osteocytes, osteoclasts, and osteoblasts form a compartment, the bone-remodeling compartment (BRC), which is separated from the rest of the marrow and which can sequester molecules that regulate the cells that remodel bone (Fig. 3.7). The signals that trigger lining cell detachment in a particular bone area are unknown. Premature apoptosis of osteocytes has been shown to precede osteoclast accumulation and resorption, which raises the possibility that osteocytes release molecules that induce lining cell retraction facilitating access of osteoclast precursors to bone surfaces. However, the molecular entities responsible for this purported osteocytic function remain unknown. As discussed earlier, osteocytes express M-CSF, which stimulates proliferation of preosteoclasts, and RANKL, the master cytokine inducer of osteoclast differentiation, both of which could reach the BRC. Growth factors released from the bone matrix upon resorption, in turn, stimulate osteoblastogenesis. It is also likely that osteocyte-derived sclerostin reaching the BRC through the canalicular system and secretion of the other Wnt antagonist Dkk-1 by osteocytes as well as osteoblasts influence the rate of bone formation, providing an additional level of control of osteoblast activity. Based on these lines of evidence, the BRC might provide a supportive environment for differentiation of osteoclast and osteoblast progenitors. Thus, regulation of the bone-remodeling rate by hormonal and mechanical stimuli could be accomplished by controlling the balance between resorption and formation within the BRC through the regulation of osteocytic molecules, including sclerostin, RANKL, and OPG.

**GROWTH, MODELING, REMODELING, AND REPAIR**

Four dynamic processes are involved in skeletal development and adaptation. These are defined by the relationship of bone resorption and bone formation to each other (Table 3.1). These mechanisms include a coordinated system that first involves the activation (A) of cell populations followed by the resorption (R) of preexisting tissue and/or the formation (F) of new or replacement tissue. Bone growth serves to increase bone mass through bone formation. Resorption of bone is not part of the growth process, and the function of growth is only to increase mass, not to adapt the developing structure to its mechanical needs. Growth can occur on a substrate but may involve ossification directly from fibrous tissue (intramembranous bone formation) or by formation of a model with cartilage first and then replacement of the cartilage with bone (endochondral ossification). Modeling uses the tissue formed during the growth process to further increase bone mass and to shape its geometry to mechanical needs. Modeling occurs through the activation of cells followed by either formation or resorption. Formation and resorption are coordinated processes in modeling but do not occur sequentially on the same surface of bone. Remodeling, on the other hand, is defined by the sequential processes on the same bone surface of activation–resorption–formation (the A-R-F sequence). The function of remodeling is bone maintenance, not increase in bone mass, and the removal of microdamage. Bone’s repair function, which restores its mechanical properties after a complete fracture or trabecular microfracture, usually occurs through the process of endochondral ossification, which forms a cartilage callus that also includes woven bone to bridge the fracture gap. This is eventually replaced through remodeling with replacement by lamellar bone.

Growth, modeling, and remodeling are present concurrently in all growing children. When skeletal maturity is reached, growth naturally stops. Modeling slows down or stops but may still be present at a reduced rate on trabecular...
surfaces and on the periosteal surface of the bone. At maturity, the predominant process is bone remodeling, which maintains the bone that has been formed and repairs microscopic damage that may be sustained in bone during normal daily activities. Dysfunction in the remodeling process is associated with the loss of bone found in osteoporosis.

GROWTH

Growth of bone occurs through two different skeletal processes, one involving formation of bone from fibrous membranes and the other involving the formation of a skeletal anlage, or model. Intramembranous bone formation occurs at centers of ossification via direct mineralization in highly vascular fibrous tissues through the action of mesenchymal cells. The calvaria of the skull is the best example of intramembranous bone formation, with the individual bones of the skull acting as centers that eventually grow together at the sutures. Apposition of bone on the periosteal surface of long bones also occurs through intramembranous ossification.

In the long bones, development generally occurs by the initial condensation of mesenchyme or hyaline cartilage in the form of the eventual skeletal structure. This cartilage model mineralizes over time and becomes detectable as a primary center of ossification. Some bones are formed from a single ossification center, although most of the long bones form secondary centers of ossification at the ends (epiphyses), which eventually fuse to the bone that developed from the primary ossification center (diaphysis) (Fig. 3.2). The secondary centers allow growth to occur at a cartilaginous growth plate until skeletal maturity in the late teens or (for the vertebral bodies) in the early part of the third decade of life. The growth plate slowly converts into primary spongiosa that becomes remodeled into lamellar trabecular plates in the metaphysis of the bone.

MODELING

Long bones must grow both in length and in diameter (Fig. 3.8). At the ends of the long bones—near the epiphyses—growth of bone demands that

![The Metaphyseal Cutback That Occurs Through Modeling Processes During Growth to Shape the Bone](http://radiologyme.com/)

**Fig. 3.8** During growth, metaphyseal bone is removed by modeling processes to shape the bone. Subsequent enlargement of the diaphysis occurs through direct peristomal apposition, which is often accompanied by resorption on the endocortical surface to enlarge the marrow cavity. Bone can also change its location and curvature through “drift.” Arrows indicate the direction of drift, with smaller circular cells representing osteoblasts and bone formation and larger ellipsoidal cells representing osteoclasts and bone resorption. (From Martin RB, Burt DB, Sharkey NA. Skeletal tissue mechanics. New York: Springer, 1998, p. 62, with permission.)

...the wider joint surface continually be reshaped and narrowed as it moves down into the metaphysis and diaphysis. Modeling is a continuous and prolonged process—unlike remodeling, which is episodic—and involves a coordinated process of bone resorption on some surfaces, while other surfaces undergo bone formation. Bone modeling occurs on both periosteal and endocortical envelopes and molds bone shape while allowing for expansion of the marrow cavity and periosteal diameter of the diaphysis. At the metaphysis, this occurs by osteoclastic resorption on the periosteal surfaces. As growth continues, however, bone is added to the periosteal surface by osteoblasts and simultaneously removed from the endocortical surface by osteoclasts. This increases whole-bone diameter and expands the marrow cavity, necessary for the formation of blood. It serves a second purpose: to increase the mechanical strength of the bone while at the same time not increasing its mass or weight at the same rate. Bone curvature is also adjusted during growth through a process known as drift, in which the periosteal surface on one side of the bone undergoes apposition while the opposite periosteal surface undergoes resorption. Likewise, different portions of the endocortical surface form or resorb bone in coordination to maintain the cortical thickness of the diaphysis.

REMODELING

The quantum concept of bone-remodeling states that bone is replaced in packets through the coupled activity of osteoclasts and osteoblasts. Coupling between osteoclasts and osteoblasts is the reason that it was difficult for so many years to control the processes involved in bone loss. When bone resorption is also suppressed because these activities are linked by intercellular signaling mechanisms that are not fully understood. In remodeling, resorption and formation are coupled but in fact may not be balanced. Coupling and balance are not the same; whereas balance refers to the relationship between the amount of bone resorbed and the amount formed, coupling denotes only that the processes are linked in some way. Resorption and formation are in balance in the healthy skeleton, but when these are out of balance, the amount of bone that is resorbed can be either greater or less than the amount that is subsequently formed. Thus, even though cells are coupled, bone can be lost or added in several different ways based on the altered balance of resorption and formation. In activity, one almost never finds a balance in favor of bone formation in a remodeling system, although this has been shown to occur with anabolic treatments for osteoporosis, such as the intermittent administration of recombinant human PTH(1-34) (Teriparatide). More often, the balance is in favor of resorption. This is the case in osteoporosis, in which global resorption is increased but formation at each of the erosion sites is normal or reduced, which leads to a deficit in bone mass.

This coupled system is termed a bone multicellular unit (BMU) because different cell populations are involved. A BMU typically consists of about 10 osteoclasts and several hundred osteoblasts. When cut in longitudinal section, the BMU shows the sequential aspects of the A-R-F system and the various mechanical relations that are involved (Fig. 3.3). Each of the phases in this sequence is location, magnitude, and rate specific, so that alterations in the magnitude or timing of one can produce morphologic features characteristic of specific skeletal abnormalities (Fig. 3.9). Activation is initiated by chemical or mechanical signals but actually involves a series of events that include recruitment of precursor cells, differentiation and proliferation of cells, and migration to the site of activity. In humans, these processes take about 5 to 10 days. Bone resorption by mature osteoclasts takes about 3 weeks at a given site, although osteoclasts moving longitudinally through bone at a rate of about 40 µm/day may live much longer than this. There is a period of reversal during which there is neither bone resorption nor formation; this may represent a period like the activation period during which osteoclasts are undergoing differentiation and proliferation from their precursors. This is followed by a period of bone formation that lasts about 3 months. As unmineralized bone—or osteoid—is laid down, it subsequently begins to mineralize quickly at first, and then more slowly over the following year. This sequence of events occurs on all four skeletal envelopes (periosteal, endocortical, trabecular, and intracortical).

Changes in bone mass can occur simply through changes in activation frequency. Changes in activation frequency may lead only to transient changes in bone mass if resorption and formation are in balance. Early losses of bone mass solely caused by increased activation frequency may resolve after several months, as newly resorbed sites are filled. Likewise, bone mass may not change because some aspect of the recruitment, proliferation, migration, or differentiation of either osteoclasts or osteoblasts is interrupted. These changes may be manifested as alterations of resorption—formation balance but may be caused by activation defects during cell maturation.